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ORAL ABSTRACT PRESENTATIONS

Parallel Session 1A: Social Hierarchies

O-001

Racial Disparities in the Association Between Birthweight in the Full Term Infant and High Blood Pressure at Age 7 Years: Results from the United States Collaborative Perinatal Project Anusha H. Hemachandra, Mark A. Klebanoff, Susan L. Furth. Department of Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, MD and the Division of Epidemiology, Statistics, and Prevention Research, National Institute of Child Health and Human Development, Bethesda, MD

Background. Blood pressure has been inversely associated with birthweight (BW) in studies worldwide. Only a few relatively small studies have included African Americans. We report here on a large biracial Unites States (US) cohort. **Methods.** The Collaborative Perinatal Project (1959-1974) prospectively studied 58,960 pregnant women and their offspring at 12 US sites. 40,901 of these children were followed until age 7 years. For this post-hoc analysis, all full term white or black children with complete follow-up and without kidney or heart disease were included. We evaluated the effect of BW and other maternal and child characteristics on systolic (SBP) and diastolic (DBP) blood pressure at age 7 years. **Results.** Of the 29,710 mother-child pairs, 47.7% were black. Mean BW was slightly lower for black compared to white infants (3.14±0.48 kg vs 3.32±0.46 kg, p<.001). Mean BMI at 7 yrs was slightly lower for black vs white children (15.8±2.0 vs 16.3± 2.0, p<.001). Compared to whites, black mothers were less likely to smoke (41 vs 52%), were more anemic (23 vs 7%), and were more likely to live in poverty (72 vs 39%). In linear regression, there was a significant interaction between race and BW in predicting SBP (p=.002). In bivariate analysis, BW was positively associated with SBP (ß=0.87) and DBP (ß=1.14) in black children (P<.001) but was not associated with either in white children. When maternal factors such as poverty, educational level and anemia during pregnancy were added to the model, BW remained a significant positive predictor of SBP (ß=0.89, p<.001) in blacks but not in whites (ß= 0.02, p= 0.17). When BMI at 7 yrs was added to the model, the relationship between BW and SBP became non-significant in blacks, and inverted and became significant in whites (β=-0.97, p<.001) **Conclusions.** In a large biracial US sample, the association between BW and SBP differed between black and white children. The etiology of IUGR-associated hypertension appears to be race-sensitive, and therefore future studies of racial disparities in the "Barker hypothesis" may help to elucidate the physiologic mechanism underlying the fetal programming of hypertension.

O-002

Relation of Maternal Low Birth Weight to the Racial Disparity in Pregnancy Outcome: A Population-Based Study James W. Collins Jr., Dyan Simon, Shilpa Vyas, Michelle Pierce, Nikhil Prachand, Richard J. David, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611 USA, and Department of Pediatrics, John H. Stronger Hospital of Cook County, Chicago, IL 60612 USA.

Background. This study sought to determine the relationship between maternal birth weight, infant intrauterine growth retardation, and prematurity. **Methods.** Stratified and logistic regression analyses were performed on a dataset of computerized Illinois vital records of African-American (N=61,849) and White (N=203,698) infants born between 1989-1991 and their mothers born between 1956-1975. **Results**. Race-specific rates of small-for-gestational age (weight-for-gestational age < 10^{th} percentile) and preterm (< 37 weeks) infants rose as maternal birth weight declined. The adjusted (controlling for maternal age, education, marital status, prenatal care utilization, and cigarette smoking) odds ratio (ninety-five percent confidence interval) of small-for-gestational age for maternal low birth weight (<2500 grams) among African-Americans and Whites were 1.7 (1.1.4-1.9) and 1.8 (1.7-2.0), respectively. The adjusted odds ratio (ninety-five percent confidence interval) of prematurity for maternal low birth weight (<2500 grams) among African-Americans and Whites were 1.6 (1.3-1.9) and 1.3 (1.0-1.6), respectively. The population attributable risk of maternal low birth weight for infant intrauterine growth retardation among African-Americans exceeded that of Whites: 8.6% versus 4.2%, p< 0.05. The population attributable risk of maternal low birth weight for infant prematurity for African-Americans and Whites were 7.5% and 1.7%, respectively. **Conclusions.** Maternal low birth weight is a risk factor for infant intrauterine growth retardation and prematurity among African-Americans independent of maternal risk status during pregnancy; it is a risk factor for infant intrauterine growth retardation among Whites. A greater proportion of growth retarded and premature African-American (compared to White) infants are attributable to maternal low birth weight.

O-003

Social and Biological Determinants of Reproductive Careers Among 12,168 Swedish Men and Women Born 1915-1929: The Uppsala Birth Cohort Multigenerational Study Ilona Koupil, Denny Vågerö, Bitte Modin, Liisa Byberg, Rawya Mohsen, Johan Fritzell, Hans Lithell and David A. Leon; Centre for Health Equity Studies (CHESS), Stockholm University/Karolinska Institute, Stockholm, Sweden; Department of Public Health and Caring Sciences, Uppsala University, Uppsala, Sweden; Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK

Background: Health inequalities and social determinants of health are a cause of much concern in all societies today. In order to contribute to our understanding on how health inequalities are transmitted from one generation to the next, we investigated social and biological correlates of reproductive careers in a cohort of Swedish men and women followed from birth to old age. **Methods:** We combined existing data on all men and women born in Uppsala Academic Hospital from 1915-1929 (the Uppsala Birth Cohort: UBCoS) with information on their descendants through linkage to the Swedish Multigeneration Registry and other routine registers. The data base contains information about families spanning over up to five generations and includes 12,168 UBCoS men and women born 1915-1929 who were resident in Sweden in 1947, their 20,397 biological children; 36,407 grandchildren and 12,862 great grandchildren born up to 2002. Information on the cohort members' birth characteristics and their families' social characteristics at the time of the cohort members' birth between 1915 and 1929 was abstracted from obstetric records. **Results:** 21.4% of UBCoS men and 18.2% of women remained childless. Among those who became biological parents, men had on average 2.31 (range 1-12) children and 4.15 (range 0-26) grandchildren, women had on average 2.30 (range 1-13) children and 4.32 (range 0-34) grandchildren. The probability of becoming a parent or a grandparent was strongly dependent on social and biological characteristics of the cohort members at birth in a gender specific pattern. Men born into families of lower social class, to unmarried mothers, men of low birth weight for gestational age, high birth order and those born preterm were the least likely to become parents. In analyses adjusted for period, age, mother's parity and social characteristics of their family at birth, men in the lowest compared to the highest quartile of weight for gestational age were 1.43 times (95% CI 1.20 to 1.72) more likely to remain childless. In contrast to the effects observed in men, women born to unmarried mothers and women of high birth order were more likely to become parents. Low weight for gestational age or a preterm birth was not associated with childlessness in women. **Conclusions:** Our forward-in-time data base is a unique source of information on social, reproductive and health careers of three generations of Swedish men and women. It shows strong and complex associations between social, biological and reproductive characteristics, all of which are likely to contribute to intergenerational effects on health and its social distribution.

O-004

Association Between Number of Siblings and Cause-specific Mortality in the Glasgow Alumni Cohort Study Galobardes B[1], McCarron P[2], Jeffreys M[3], Davey Smith G[1]. [1]Department of Social Medicine, University of Bristol, Canynge Hall, Whiteladies Road, Bristol, BS8 2PR, UK; [2]The Queen's University of Belfast, UK; [3]Massey University, Wellington, New Zealand.

Background. Number of siblings has been positively related to mortality risk, particularly that from haemorrhagic stroke and stomach cancer, pointing to early life deprivation and early life infections as potential aetiological mechanisms for these associations. The objective of this study is to investigate the relation between number of siblings and adult cause-specific mortality in a historical cohort with extensive mortality follow-up. **Methods.** In a prospective follow-up of a historical cohort of students (11,756 men), attending Glasgow University between 1948 and 1968, who participated in a student health survey, clinical measures and sociodemographic characteristics, including number of siblings, were recorded. Since 1998, approximately 85 percent of the male cohort has been successfully traced through the UK National Health Service Central Register, which provides continuous updates of the date and cause of death for members of the cohort. Only men were included in this report because of the small number of women attending university from 1948-68 and the low number of female deaths. Cox proportional hazards models were used to estimate the hazard ratio (HR, 95% confidence interval) of cause-specific mortality adjusting for date of examination, father's occupational class, height, body mass index, systolic blood pressure, and smoking habit in young adulthood. Number of siblings was categorized as 0, 1 (reference group), 2, 3, and 4 or more siblings and was also analyzed as a continuous variable. **Results.** Participants' ages ranged from 16 to 30 years. Until March 2005, there have been 1,321 deaths. A higher number of siblings was positively related to lower father's social class (trend p≤0.0001), lower height (trend p≤0.0001) and lower systolic blood pressure (trend p≤0.0001), but not to body mass index (trend p=0.102), current smoking (trend p=0.214) or alcohol consumption (trend p=0.937) in young adulthood. Having four or more siblings was associated with higher all cause (HR=1.21, 95% CI: 1.01, 1.44) and respiratory disease (HR=2.12, 95%CI: 1.06, 4.24) mortality after adjusting for all available socioeconomic and disease risk factors. The HR for cardiovascular disease (HR=1.21, 95%CI: 0.92, 1.59), ischaemic stroke (HR=2.66, 95%CI: 0.53, 13.43) and prostate cancer (HR=1.71, 95%CI: 0.68, 4.29) mortality were raised but did not reach conventional statistical significance. Number of siblings was not related to haemorrhagic stroke (HR=0.60, 95%CI: 0.17, 2.09), lung cancer (HR=0.67, 95%CI: 0.29, 1.53), stomach cancer (HR=0.92, 95%CI: 0.18, 4.64) mortality or to external causes of death (HR=1.08, 95%CI: 0.49, 2.37), although confidence intervals were wide. **Conclusions.** Number of siblings was related to mortality risk in this cohort, although for some causes of death adjusting for socioeconomic circumstances and disease risk factors accounted for the observed association. However, number of siblings remained associated with all cause and respiratory disease mortality. Residual confounding due to smoking habits in early or late adulthood is unlikely to explain the association with respiratory disease

mortality given that no association was observed with lung cancer mortality. The participants in the Glasgow Alumni Cohort represent a sector of the population of generally high socioeconomic position, and are unlikely to have experienced any form of severe childhood deprivation. This may explain the lack of association with stomach cancer and haemorrhagic stroke. The role of common early childhood infections may have a role in explaining these associations.

Parallel Session 1B: Cancer and Reproductive Outcomes

O-005

Pregnancy Outcomes and Risk of Breast Cancer in the Mother Janet W. Rich-Edwards, Bernard Rosner, Graham A. Colditz, Eileen N. Hibert, Karin B. Michels, Rosalind J. Wright, and Walter C. Willett; *Harvard Medical School and Harvard School of Public Health, Boston, MA 02215, USA*

Background. First pregnancy has a profound impact on the mammalian breast. Previous studies have conflicted whether characteristics of pregnancy, such as gestation length, offspring birthweight, and offspring gender affect breast cancer risk in the mother. **Methods**. We examined these questions in the Nurses' Health Study II, using a nonlinear Poisson regression model of breast cancer incidence (Rosner model) to examine gestation length, offspring birthweight, and offspring gender for each pregnancy, accounting for age, age at menarche, age at pregnancy, parity, benign breast disease, body mass index, height, alcohol intake, and family history of breast cancer. We examined 64,623 premenopausal women age 25-54 who reported only singleton pregnancies. **Results**. For first pregnancies only, birthweight was positively and gestation length was negatively associated with subsequent risk of premenopausal breast cancer in the mother; the magnitude of the associations increased with gynecologic age at first pregnancy. For every kilogram increase in firstborn birthweight, women had an 11% (95% confidence interval, 3%-20%) higher risk of later breast cancer if the first pregnancy occurred at age 20, and a 22% (6%-40%) increased risk if first pregnancy occurred at age 30. Women whose first pregnancy was delivered preterm (≤37 gestation weeks) had a 7% (1%-12%) increased risk of later breast cancer if the preterm birth occurred at age 20, and a 13% (2%-24%) increased risk if the preterm birth occurred at age 30, compared with women whose first pregnancies went to term. Neither birthweight nor gestation length of subsequent pregnancies was associated with maternal breast cancer risk. Offspring gender had no association with maternal risk of breast cancer. **Conclusions**. For first, but not subsequent pregnancies, higher offspring birthweight and shorter gestation were associated with increased risk of premenopausal breast cancer.

O-006

Offspring Birth Size and Maternal Breast Cancer Risk Paul R. Romundstad, Tom I. Lund Nilsen, Lars Vatten; *Department of Public Health, Norwegian University of Science and Technology, 7006 Trondheim, Norway*

Background: The positive association between birth size and breast cancer risk may be mediated through intrauterine levels of hormones and growth factors. However, since adult body height is positively associated with breast cancer risk, and since parental body size influence offspring birth size, one may also suggest that shared genetic or lifestyle factors could mediate the association. Therefore, mothers who give birth to large babies may be expected to be at an increased risk for breast cancer. **Methods:** In a prospective study of 751,571 women registered in the Medical Birth Registry of Norway with a singleton first birth between 1967 and 1998, we examined the association between the first offspring birth size and subsequent risk for breast cancer in the mother. Women with missing or unrealistic combinations of gestational age, birth length and birth weight in the first birth were excluded from the analyses (n=75,190) as well as women with a cancer diagnosis before start of follow-up (n=1,324). This left 675,057 women for the analyses. Follow-up was from date of first birth until the first diagnosis of breast cancer, another cancer, emigration, death from other causes than cancer, or to the end of follow-up (31 December 2002), whichever occurred first. We used Cox regression with attained age as the time variable and controlled for year of birth of the mother, parity, age at first birth, sex of offspring, marital status and gestational age. **Results:** Among 8,914 incident cases of breast cancer, we found no significant associations between offspring birth dimensions and breast cancer risk, RR=1.01 (95% confidence interval (CI) 0.98-1.04) per sd (2.7 cm) increase in birth length, RR=1.00 (95% CI 0.98-1.03) per sd (560 g) increase in birth weight, and RR=1.00 (95% confidence interval (CI) 0.97-1.02) per sd (2.6 kg/m^3) increase in ponderal index. Categorization of the birth size variables or mutual adjustment of birth length, birth weight, and ponderal index did not change the results. Moreover, the findings were similar in analyses of pre- and postmenopausal breast cancer (below and above 50 years of age), or stratified by age at first pregnancy (below and above 30 years) or by offspring sex. Suplementary analysis stratified on localized tumours and tumours with affected lymph nodes did not change these results, and neither did the ecxlusion of women with gestational diabetes or preeclampsia. **Conclusion:** The present study does not support the hypothesis that shared genetic or lifestyle factors underly the association between birth size and breast cancer risk in adulthood.

O-007

Does High *in utero* Hormonal Environment Increase Breast Cancer Risk by Affecting Mammary Stem/progenitor Cell Populations? Jennifer Webster, Pria Anand, Bin Yu, Galam Khan, Luis E Dettin, Sonia de Assis, Ayesha Shajahan and Leena Hilakivi-Clarke; *Department of Oncology, Georgetown University, 3970 Reservoir Rd, NW, Washington, DC 20057, USA*

Background: High birth weight is associated with increased breast cancer risk, and this could reflect elevated pregnancy estradiol (E2) or leptin levels. Findings in animals show that maternal E2 exposure during pregnancy increases female offspring's mammary tumorigenesis; however, the effects of maternal leptin exposure have not been studied. More importantly, if *in utero* hormonal environment can modify later breast cancer risk, how does this happen? Findings obtained in animals suggest that *in utero* E2 exposure modifies normal mammary gland development in a manner that leads to either an increase in the number of cells that are susceptible for malignant transformation; i.e., terminal end buds (TEBs) or an increase in cell proliferation within TEBs. These structures also are likely to contain somatic stem cells that are thought to be converted to tumor stem cells. To test this hypothesis, we determined whether *in utero* exposure to E2 or leptin alters putative mammary stem/progenitor cell population. In addition, the effects of *in utero* leptin exposure on carcinogen-induced mammary tumorigenesis were studied. **Methods**: Pregnant female Sprague Dawley rats were exposed daily to vehicle, 10 μg E2 or 15 μg leptin between gestation days 10 and 20. Mammary glands of offspring were obtained on postnatal day 21 (before puberty) and day 50 (mammary gland is most susceptible for malignant transformation) and mammary tumors were induced by administering 50-day-old rats 10 mg 7,12-dimethylbenz[a]anthracene (DMBA). Stem/progenitor cell populations were evaluated by identifying side-population using Hoechst staining in flow cytometry and by counting nestin-positive cells in TEBs by using immunofluorescent technique. Nestin was chosen because it is a marker of neuronal and other somatic stem cells, and was recently identified in gene microarrays as being down-regulated in cells that over-express caveolin-1, a scaffolding protein that is expressed only in differentiated cells. Further, we recently found that *in utero* E2 exposure reduces caveolin-1 expression in the rat mammary gland. **Results**: The results indicated that similarly to estradiol, *in utero* exposure to leptin increased the number of TEBs in the mammary gland ($p<0.01$) and the susceptibility of developing carcinogen-induced mammary tumors. Specifically, mammary tumor incidence ($p<0.001$) and multiplicity ($p<0.007$) were significantly increased in the E2 and leptin offspring, when compared to the vehicle group. Examination of mammary stem/progenitor cell populations indicated that at 21 days of age no changes in stem cell populations were noted. However, at 50 days of age the mammary epithelium of the *in utero* E2 exposed rats contained more stem/progenitor cells (nestin-positive: 7.0%) than the controls (3.7%) ($p<0.04$). Further, the TEBs contained 1.7-fold higher number of stem/progenitor cells (6.1%) than the ducts and lobules (3.5%). *In utero* leptin exposure did not modify the number of these cells. **Discussion**: Our results suggest that *in utero* exposure to E2 or leptin both increase later breast cancer risk. The increase in the *in utero* E2 exposed rats may be associated with increased number of stem/progenitor cells at the time the rat is most susceptible for the initiation of breast cancer.

O-008

Prenatal Glucocorticoid Exposure Affects Reproductive Capacity and Adrenocortical Function in Mature Female Guinea Pig Offspring Elizabeth Dunn, Dawn Owen, Alice Kostaki, Stephen G Matthews, *Departments of Physiology, Obstetrics & Gynaecology and Medicine, University of Toronto, 1 King's College Circle, Toronto, Ontario M5S 1A8, CANADA.*

Background: Synthetic glucocorticoids (GC) are currently recommended for mothers at risk of preterm delivery between 24-34 weeks to promote fetal lung maturation. Evidence is emerging indicating long-term effects of repeated courses on development and function of specific organs, especially the brain. In the present study we hypothesized that repeated maternal treatment with synthetic (GC) will alter reproductive capacity and adrenocortical function in mature adult female guinea pig offspring. **Methods:** Pregnant guinea pigs (F_0) were subcutaneously injected with betamethasone (Beta 1mg/kg) or vehicle (Veh) on gestational days (GD) 40/41, 50/51 and 60/61 or left undisturbed (U). Animals were allowed to deliver naturally. Reproductive cycles (~ 16 days) were monitored in female offspring from weaning. After 140 days of age, basal salivary cortisol was determined at multiple times of the day over one reproductive cycle (days 0 (estrous), 8 & 11). On subsequent days (1, 9 & 12) females were exposed to a high frequency strobe light stress (30 min). Salivary cortisol was determined before, during and after stress exposure. After endocrine testing, females (F_1) were mated and their reproductive success and pregnancy monitored. Once pregnant, salivary cortisol was collected at multiple time points in late pregnancy. After offspring were weaned mothers (F_1) were euthanized in the luteal phase of their next cycle; blood and major organs were collected and weighed. **Results:** Basal salivary cortisol levels remained relatively low throughout the reproductive cycle in the Beta females compared to controls (Veh and U). Salivary cortisol was significantly higher ($p<0.05$) in the Beta than the Veh females following SL in the follicular phase, indicating hyperresponsiveness to stress. Female offspring that had been exposed to Beta *in utero* took significantly longer to become pregnant ($p<0.05$). In pregnancy, salivary cortisol in Beta and Veh females failed to rise after GD 40, compared to the U animals whose levels continued to rise to term. There was a trend towards a smaller litter size in Beta-exposed animals. Female (F_1) kidney and liver to total bodyweight ratios were significantly lower in Beta exposed animals

($p<0.05$) compared to Veh and U females. **Conclusion:** Prenatal exposure to synthetic GC's causes alterations in basal and stimulated pituitary-adrenal activity that change as a function of the reproductive cycle. There are also long-term changes in the renal and hepatic systems along with marked log-term effects on reproductive capacity. In addition there also appears to be an effect from the injection itself, perhaps via release of endogenous cortisol or sympathetic activity. **Supported by:** Canadian Institutes of Health Research (SGM) and Mount Sinai Hospital.

Parallel Session 1C: Endocrine Mechanisms

O-009

Increased Intramyocellular Lipid Causes Skeletal Muscle Insulin Resistance in the Young Adult Guinea Pig of Low Birthweight Julie A Owens, Melanie Tran, Irving Lee, Robyn Taylor, Dane Horton and Jeffrey S Robinson; *Department of Obstetrics and Gynaecology, The University of Adelaide, Adelaide, SA, 5005, Australia*

Background: Restricted fetal growth is now recognised as a novel and significant risk factor for diabetes and insulin resistance in later life, but the underlying defects induced to cause these conditions are poorly understood. Recent evidence has shown that restricted fetal growth is characterised by insulin resistance of glucose uptake and utilisation in skeletal muscle in young adult men and women. Insulin resistance due to other factors such as obesity, appears to result from impaired 5' AMP activated protein kinase (AMPK)/ malonyl CoA fuel sensing and activity, dysregulated lipid metabolism and accumulation of intramyocellular lipid and related metabolites, which impair insulin signalling and its targets. We therefore hypothesized that fetal growth restriction would cause skeletal muscle insulin resistance and increase intramyocellular lipids in the young adult guinea pig and that activation of AMPK to lower intramyocellular lipids would normalise this prenatally induced insulin resistance. Methods: Young adult guinea pigs of varying size at birth (n=16) had vascular catheters inserted at 100 days of age then hyperinsulinaemic euglycaemic clamps (HEC) carried out with D-2-[1-^{14}C]-deoxyglucose to measure insulin sensitivity of glucose uptake, phosphorylation and incorporation into glycogen in four muscles (in order of decreasing proportion of type 1 insulin sensitive fibres): biceps brachii, vastus lateralis, gastrocnemius medialis and biceps femoris. Intramyocellular lipid in vastus lateralis was quantified by image analysis following Oil Red O staining of cryosections. Additional young adult guinea pigs of low birthweight (n=5) were treated with AICAR (60mg/kg sc every 2 days) (5-aminoimidazole 4-carboxamine-riboside) or vehicle for 6 days then HEC carried out to measure insulin sensitivity. **Results:** Whole body and peripheral insulin sensitivity of glucose utilisation decreased with birth weight ($r=0.45$, $p=0.03$, $r=0.69$, $p<0.001$). Insulin sensitivity of glucose uptake and phosphorylation, incorporation into glycogen and their total in skeletal muscle decreased with decreasing birthweight in vastus lateralis ($p<0.1$ for all) and in all four muscles in males (biceps brachii, $r=0.97$, $p<0.001$, $r=0.92$, $p=0.002$, $r=0.96$, $p<0.0001$; vastus lateralis: $r=0.60$, $p=0.10$ for uptake and phosphorylation only; gastrocnemius, $r=0.85$, $p=0.017$, $r=0.889$, $p=0.009$, $r=0.72$, $p=0.05$; biceps femoris, $r=0.77$, $p=0.064$ for uptake and phosphorylation only). Intramyocellular lipid increased with decreasing birthweight ($r=-0.44$, $p=0.06$). Insulin sensitivity of glucose uptake and phosphorylation, incorporation into glycogen and their total also correlated inversely with intramyocellular lipid ($r=-0.58$, $p=0.03$, $r=-0.52$, $p=0.05$, $r=-0.58$, $p=0.03$). AICAR treatment increased whole body insulin sensitivity of young adult guinea pigs of low birthweight (+58%, $p<0.05$) to that seen on average and high birthweight animals. **Conclusion:** Fetal growth restriction impairs insulin sensitivity of glucose uptake, phosphorylation and incorporation into glycogen in skeletal muscle of the young adult guinea pig and this is associated with increased intramyocellular lipid. AMPK activation normalised insulin sensitivity, suggesting that dysregulated lipid metabolism and accumulation of inhibitory lipid species caused the prenatally induced insulin resistance.

O-010

Transgenerational Effects of Undernutrition in Early or Late Pregnancy on Hypothalamo-pituitary-adrenal (HPA) Function in Adult Male Guinea Pig Offspring C.E.Bertram[1], O.Khan[1], C.Loades[1], DIW Phillips[2], SG Matthews[3] M.A.Hanson[1] Centre for DOHaD, University of Southampton, UK., [2]MRC Epidemiology Unit, University of Southampton, UK, [3]Depts Physiology, Ob-Gyn & Medicine, University of Toronto.

Introduction: Epidemiological studies suggest that the risk of chronic disease in adulthood may, in part, be determined in fetal life by maternal nutrition. Studies of survivors of the Dutch Hunger Winter show that the timing of the nutritional insult *in utero* is crucial in determining the risk of various pathologies in later life. It has recently been shown that short periods of prenatal stress or acute (48 hr) food deprivation in late gestation affect HPA axis activity in adult guinea pig offspring. We determined the impact of nutritional insult in early or late gestation on HPA function in first (F_1) and second (F_2) generation male offspring. **Methods:** Adult (~450 g bodyweight) female guinea pigs (F_0) were mated. Animals were restricted to 70% average *ad lib* food intake/kg bodyweight from either days 1-35 (early – ER) (n=17) or days 36-70 (late – LR) (n=17) of gestation. Control (C) animals were fed standard chow *ad lib* throughout gestation (n=14). Female offspring (F_1) from each group were mated (~ 450g), but all fed *ad lib*. Adult male offspring (F_1 & F_2) were chronically catheterised and HPA axis function evaluated using dexamethasone (dex) suppression followed by CRH challenge. **Results:** Basal cortisol and ACTH concentrations were elevated in both LR_{F1} and LR_{F2} adult male offspring ($p<0.05$) and were not totally suppressed by administration of DEX at t=240 mins in LR_{F1} ($p<0.05$) *vs.* C offspring. The area under the curve (AUC) of the ACTH ($p<0.01$) and cortisol ($p<0.05$) response to CRH was greater in the LR_{F1} group. In the F_2 generation, the LR_{F2} cortisol response to CRH was increased ($p<0.05$), compared to controls, while ER_{F2} cortisol was decreased ($p<0.05$); there were no significant differences in ACTH responses.

Table 1: Basal plasma ACTH and cortisol (at t = 0min) and responses to suppression by DEX (at t = 240 min) and stimulation (AUC) by CRH. Group data (mean ± SEM) were analysed using one-way analysis of variance (ANOVA) followed by Bonferonni post-hoc comparison. ** = $p<0.01$, * = $p<0.05$ *compared with controls*

	Basal		suppressed		CRH challenge		Basal		suppressed		CRH challenge	
	ACTH pg/ml	sem ±	ACTH pg/ml	sem ±	ACTH	sem ±	cortisol ng/ml	sem ±	cortisol ng/ml	sem ±	cortisol	sem ±
C_1	56.0	7.2	12.5	3.8	243.3	12.6	24.4	2.4	1.0	0.1	85.5	16.9
ER_1	70.0	6.6	16.4	4.9	248.0	12.9	36.1	3.3	1.0	0.2	79.2	9.7
LR_1	**81.0***	5.9	**31.0***	4.1	**322.5****	9.7	**46.2***	2.1	7.0*	0.5	**108.0***	10.2
C_2	50.0	3.1	11.7	1.7	239.0	2.7	33.4	4.2	4.4	0.9	116.7	8.2
ER_2	**69.0***	5.1	10.4	3.1	225.5	3.7	42.9	5.1	5.1	1.1	**88.0***	6.3
LR_1	**71.7***	3.5	9.6	5.2	244.0	4.4	**49.0***	4.5	8.7	0.7	**136.9***	7.5

Conclusions: We demonstrate for the first time that undernutrition has transgenerational effects on the HPA axis. Undernutrition in late gestation results in offspring which in adulthood have increased basal ACTH/cortisol together with reduced sensitivity to DEX suppression and higher CRH responses. In turn their offspring (in the absence of a nutritional challenge) have increased basal ACTH/cortisol concentrations and increased CRH responses in adulthood. These findings of non-genomic transmission of a phenotype known to be linked with human disease have profound implications for understanding the mechanisms underlying early origins of such disease. This research was supported by a grant from the British Heart Foundation (MAH & SGM)

O-011

Lower Placental 11ß-HSD2 Activity is Associated with Higher Blood Pressure in Childhood Susanna Y. Huh,[1,2] Ruth Andrew,[3] Janet W. Rich-Edwards,[2] Ken P. Kleinman,[2] Jonathan R. Seckl,[3] Matthew W. Gillman[2]; [1]*Division of Gastroenterology and Nutrition, Children's Hospital Boston, Boston, MA, USA, 02115,* [2]*Department of Ambulatory Care and Prevention, Harvard Medical School/Harvard Pilgrim Health Care, Boston, MA, USA 02215, and* [3]*the School of Molecular and Clinical Medicine, University of Edinburgh*

Background: Placental 11-beta-hydroxysteroid dehydrogenase type 2 (11ß-HSD2) inactivates active circulating maternal cortisol (F) to inert cortisone (E). Animal experiments have shown that decreased activity of 11ß-HSD2 permits increased access of active maternal glucocorticoids to the fetus, programming both lower birth weight and offspring hypertension. To our knowledge, this is the first study in humans to examine the relationship between placental 11ß-HSD2 activity and blood pressure. **Objective:** To examine the relationship between 11ß-HSD2 activity, as measured by the ratio of cortisone (E) to total glucocorticoids (E+F) in venous cord blood, and blood pressure (BP) at age 6 months and 3 years. **Methods:** We measured cortisol and cortisone by RIA in umbilical venous cord blood of 362 newborns from Project Viva, a prospective US cohort study of pregnant women and their offspring. We measured BP up to 5 times using an automated device when the children were age 6 months (n=362) and age 3 years (n=138). Using multivariable mixed effects models to control for BP measurement conditions, maternal and infant characteristics, we examined the association between E/(E+F) ratio and childhood BP. **Results**: Mean cortisol was 347.0 nmol/L and mean cortisone was 240.9 nmol/L. Mean E/(E+F) ratio was 43.4% (20.7% – 67.2%, 1st to 99th %ile). Mean systolic BP was 90.2 mm Hg at age 6 months, and 94.1 mm Hg at age 3 years. At age 6 months, we did not find an association between E/(E+F) ratio and systolic or diastolic BP. At age 3 years, each 10% decrement in E/(E+F) was associated with a 1.3 mm Hg increase in systolic BP (95% CI 0.0, 2.5). We found a similar effect estimate for diastolic BP at 3 years (1.2 mm Hg per each 10% decrement in E/(E+F) ratio, 95% CI 0.2, 2.2). E/(E+F) ratio was not associated with birth weight for gestational age z-score.
Conclusion: Decreased E/(E+F) ratio, a marker of placental 11ß-HSD2 activity, predicts increased blood pressure at age 3 years. Our data suggest that increased fetal exposure to glucocorticoids may program later blood pressure in humans.

O-012

Leptin Mediated Mechanism for Programmed Obesity in Intrauterine Growth Restricted Newborns Mina Desai, Dave Gayle, Guang Han, Ederlen Casillas, and Michael G. Ross; *Department of Obstetrics and Gynecology, David Geffen School of Medicine at UCLA and LABioMed at Harbor-UCLA Medical Center, Torrance, CA 90502, USA*

Background: Environmental factors such as maternal nutrition, acting via epigenetic mechanisms during critical fetal and/or neonatal developmental periods, play a crucial role in the development of adult obesity. We have previously shown that maternal nutrient restriction results in intrauterine growth restricted (IUGR) newborns with hypoleptinemia evident at one day of age. Those offspring permitted rapid catch-up growth, by cross fostering to normal lactating dams, demonstrate evidence of increased

body weight, body fat and hyperleptinemia, at nine months of age. The fact that hyperleptinemia precedes the development of increased body fat suggests a key role of leptin mediated-obesity. Leptin binding to its full-length receptor (OB-Rb) activates intracellular signaling pathways via phosphorylation of several tyrosine residues of the Janus kinase family (JAK2), which leads to the translocation of STAT3 to the nucleus, where it regulates gene transcription. We hypothesized that an underlying mechanism of programmed offspring obesity involves defects in hypothalamic leptin receptors or postreceptor signaling. We sought to determine hypothalamic mRNA expression of leptin receptor (Ob-Rb) and protein expression of STAT3 in IUGR offspring predisposed to adult obesity. **Methods**: Pregnant Sprague Dawley rats and offspring were studied. Control dams (n=6) received ad libitum food, whereas study dams (n=6) were 50% food-restricted from pregnancy day 10 to 21 to produce IUGR newborns. Blood samples and brain were collected from 1 day old newborns and processed for plasma leptin and ghrelin levels (RIA), and hypothalamic Ob-Rb mRNA levels (real-time RT-PCR) and STAT3 protein expression (Western Blot followed by densitometric analysis). Data for real-time RT-PCR is presented as threshold cycle (C_T) normalized to ß-actin (i.e., higher amounts of signal have correspondingly lower C_T values). **Results:** At 1d of age, pups from food-restricted dams had lower body weights (6.0±0.3 vs 7.1±0.3 g, p<0.01), decreased plasma leptin (0.66±0.03 vs 1.63±0.12 ng/ml, p<0.001) and increased plasma ghrelin levels (0.43±0.03 vs 0.26±0.02 ng/ml, p<0.01) than control pups. IUGR pups showed a 4-fold increase in Ob-Rb mRNA expression (12±0.2 vs 14±0.1 C_T, p<0.01) and elevated STAT3 protein levels (1367±54 vs 630±67 AU, p<0.001) as compared to pups of control dams. **Conclusion:** Maternal food-restriction during pregnancy results in IUGR pups which exhibit hypoleptinemia, and upregulation of leptin receptor (Ob-Rb) and STAT3 expression at 1 day of age. These results indicate evidence of peripheral and central leptin deficiency in IUGR pups, and suggest that alterations in leptin receptor and/or post-receptor signaling may contribute to programmed obesity in IUGR offspring.

Parallel Session 1D: Animal Models

O-013

11ß-Hydroxysteroid Dehydrogenase Ype 2 (11ß-HSD2) Knockout Mice as Model of Prenatal Glucocorticoid Programming

C.T. Abrahamsen, R.N. Carter, J.R.Seckl & M.C. Holmes; Endocrinology Unit, Centre for Cardiovascular Science, The Queens Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh EH16 4TJ, UK

Introduction: 11ß-Hydroxysteroid dehydrogenase type 2 (ß2) inactivates glucocorticoids by conversion of corticosterone to 11-dehydrocorticosterone in rodents. ß2 is highly expressed in both the placenta and developing brain regions, and thus is believed to provide a barrier and protect the developing fetus from overexposure to maternal glucocorticoids. Studies have shown that prenatal exposure to synthetic glucocorticoids or stress, reduce birth weight and cause lifelong pathophysiology, including anxiety-related behaviour – termed early-life programming. The protective importance of ß2 is underlined by reduction in ß2 activity by ß2 polymorphisms, enzyme inhibition, or targeted deletion of ß2 gene, causing low birthweight and other programming effects. However, all of these conditions involve an 'unwell' mother suffering from the Syndrome of Apparent Mineralocorticoid Excess. Maternal postnatal behaviour has an effect on offspring development, hence we have circumvented this problem by carrying out heterozygous matings, producing same-litter mice with varying levels of ß2 expression, therefore correcting for any differences in nurturing behaviour. The aim was to determine whether loss of ß2 results in lifelong programming of anxiety-related behaviour and hypothalamo-pituitary-adrenal (HPA) axis, independent of any variation in maternal care or pathophysiology. **Methods:** Mice heterozygous for the ß2 gene deletion (ß2+/-) were mated and pups weighed at birth. Behavioural analysis was performed on the three offspring genotypes (ß2-/-, ß2+/-, ß2+/+) using the elevated plus maze (EPM), open field apparatus (OF) and modified light/dark box (L/D). Plasma corticosterone levels were measured by RIA under basal and post restraint conditions. Results are expressed as mean±SEM and were compared using one-way analysis of variance with Student-Newman-Keuls post-hoc test, where appropriate. **Results:** ß2-/- were smaller at birth than ß2+/+, whilst ß2+/- birthweights were in between (ß2-/- 1.14±0.02g; ß2+/- 1.24±0.02g; ß2+/+ 1.33±0.03g; n=26-27, P<0.01). ***Males: EPM*** ß2-/- entered the open arms less (ß2-/- 8.1±1.0; ß2+/- 14.4±0.8; ß2+/+ 15.1±1.6; n=8-16, P<0.001) and spent less time on them (ß2-/- 48.5±9.1s; ß2+/- 86.1±9.7s; ß2+/+ 79.0±8.2s; n=8-16; P<0.05). ***OF*** Total percentage of crossings within the more anxiogenic inner zone did not differ, although ß2-/- made fewer crossings during the first minute (ß2-/- 10.3±1.9%; ß2+/- 20.4±3.4%; ß2+/+ 26.9±5.8%; n=7-15, P<0.05). ***L/D*** ß2-/- made fewer crossings (ß2-/- 12.7±2.8; ß2+/- 18.9±1.2; ß2+/+ 20.8±1.8; n=6-12, P<0.05) and spent less time in the light compartment (ß2-/- 75.7±14.9s; ß2+/- 108.2±9.3s; ß2+/+ 114.8±6.4s; n=6-12, P<0.05). ***Females: EPM*** ß2-/- made fewer entries into the open arms (ß2-/- 12.9±0.9; ß2+/- 16.7±1.1; ß2+/+ 16.8±1.2; n=5-11, P<0.05), although the open duration was not different. ***OF*** The total proportion of inner zone crossings was unchanged, although was decreased during the first minute (ß2-/- 5.7±1.3%; ß2+/- 11.1±2.0%; ß2+/+ 14.4±2.7%; n=5-8; P<0.05). ***L/D*** No differences were found between genotypes. All behavioural data are consistent with ß2-/- mice showing increased anxiety-related behaviour in both sexes. There was no difference in basal and post restraint plasma corticosterone levels between genotypes, suggesting there may not the programming of the HPA axis in the ß2-/- mice. **Discussion:** Our data show that loss of ß2 results in a programmed anxious phenotype, albeit with unaltered corticosterone levels under normal or stress conditions. This anxious phenotype is apparent in the absence of any variation in maternal behaviour or pathophysiology. Whilst mice heterozygous for the ß2 gene deletion displayed reduced birthweight, they were not different from the wild type in our other analyses, suggesting a greater decrease in ß2 activity is necessary before deleterious effects in programmed anxiety-related behaviour are observed.

O-014

Transgenerational Effects of Pre-natal Undernutrition on Cardiac Morphology

OA Khan[1,2], C Bertram[2],C Loades[2], J Boullin[1,2], SG Mathews[3], SK Ohri[1] & MA Hanson[2] ; 1. Wessex Cardiothoracic Centre, Southampton General Hospital, UK; 2. Centre for Developmental Origins of Health and Disease, University of Southampton, UK; 3. Department of Physiology, Obstetrics and Gynaecology University of Toronto, Toronto, Canada.

Background: We have previously utilised an animal model to illustrate that early gestation undernutrition leads to left ventricular (LV) hypertrophy and hypertension in the F_1 generation [1]. We have now extended the study to investigate whether this phenomenon is seen in the next (F_2) generation.
Methods: Individually housed virgin female guinea pigs were mated and assigned to two dietary groups: **A.** Control (C) - standard chow ad lib **B.** Early-restricted (ER) - 70% of the average daily food intake/kg for the first half of gestation. Sows and their offspring were fed standard chow ad lib from birth onwards. The F_1 females were then mated and fed *ad libitum* throughout pregnancy. The F_1 and F_2 male offspring underwent echocardiography 12 weeks following birth in order to characterise their cardiac function. The right carotid artery was then catheterised under general anaesthesia, and after 7 days recovery, blood pressure was measured over a standardised four hour period. Data is expressed as mean ± sem. (*p<0.05 compared to controls).

Results:

	Control	ER (F_1)	ER (F_2)
LV Diameter in diastole (mm)	11.6 ± 0.3	11.9 ± 0.33	10.8 ± 0.2
LV Diameter in systole (mm)	8.75 ± 0.3	8.8 ± 0.32	7.9 ± 0.2
Fractional shortening	24.6 ± 1.1	25.5 ± 1.3	26.7 ± 1.9
Mean LV wall thickness (mm)	2.75 ± 0.1	**3.30 ± 0.09***	**3.60 ± 0.1***
Corrected LV Mass (mg/g)	4.58 ± 0.42	**5.86 ± 0.31***	**5.30 ± 0.1***
Mean arterial blood pressure	64.8 ± 1.7	**72.6 ± 1.90***	63.8 ± 2.2

Table 1

As shown in table 1, F1 animals in early restricted group showed elevated baseline mean arterial blood pressure and increased LV wall thickness and mass as compared to control animals. In addition, F2 animals whose grandmothers had early nutrient restriction showed increased LV wall thickness and mass, but were normotensive. **Conclusions:** Early nutritional restriction in guinea pigs is associated with cardiac hypertrophy in both the F1 and F2 generations. The fact that hypertrophy occurs in the F2 generation, even in the absence of a nutrient challenge in the F1 females, demonstrates that fetal undernutrition can induce transgenerational non-genomic effects on cardiac morphology. References: **[1] Khan OA et al. *Circulation* 2004;** 110(7) III: **102** *Supported by the British Heart Foundation (MAH, SGM) and HOPE(OK).*

O-015

Early Gestation Dexamethasone-Exposure Programs Tissue-Specific Alterations in Reactive Oxygen Species Production

Robert D. Roghair[1], Fred S. Lamb[1], Francis J. Miller Jr.[2], Jeffrey L. Segar[1]; Department of Pediatrics[1] and Department of Internal Medicine[2], Carver College of Medicine, University of Iowa, Iowa City, IA USA

Background: Fetal programming of hypertension and coronary artery disease may be a consequence of glucocorticoid-induced alterations in tissue-specific renin-angiotensin systems (RAS). These alterations have been most pronounced within the kidney, brain, and coronary circulation. Because angiotensin II (ANG II) is the prototypical agonist for endothelial NAD(P)H oxidase-dependent reactive oxygen species (ROS) production, programmed RAS upregulation may lead to enhanced tissue-specific oxidative injury. **Hypothesis**: Early gestation dexamethasone exposure (1) enhances coronary artery RAS-dependent ROS production and (2) increases tissue-specific oxidative injury. **Methods**: Dexamethasone (dex; 0.28 mg/kg/d IV for 48 hours) was administered to pregnant ewes at 27-28 days gestation (term being 145 d). Basal and NADPH-stimulated ROS production was measured by lucigenin-enhanced chemiluminescence on coronary and mesenteric arteries harvested from dexamethasone-exposed and age-matched control lambs both at 125 days gestation (n = 6) and 4 months following delivery (n = 7). Postnatal superoxide production was also assessed following 4 h incubation with low (10^{-9}M) or high (10^{-7}M) concentrations of ANG II. Oxidative injury was assessed in the postnatal sheep by measurement of aconitase activity (decreased following protein oxidation) and lipid peroxidation. **Results**: Coronary but not mesenteric arteries from dex-exposed fetuses exhibited diminished basal, but enhanced NADPH-stimulated ROS production (both $P < 0.05$). Although basal and NADPH-stimulated ROS production was similar in the dex-exposed and control postnatal coronary and mesenteric arteries, incubation with 10^{-7}M ANG II significantly increased NAD(P)H-dependent ROS production exclusively within the dex-exposed coronary arteries. Finally, although early gestation dex-exposure did not alter lipid peroxidation, it was associated with increased renal and cerebral cortex aconitase activity (both $P < 0.05$). **Conclusion**: Early gestation dex-exposure is associated with decreased (1) fetal coronary artery ROS production and (2) postnatal kidney and brain oxidative protein injury. Following addition of the NAD(P)H oxidase substrate NADPH, there was a dramatic vessel specific increase in

ROS production within both the prenatal and ANG II-incubated postnatal coronary arteries that had been previously exposed to dexamethasone. This dysregulated ROS production may contribute to progressive coronary dysfunction when an adverse postnatal environment (such as RAS-mediated hypertension) is superimposed upon prenatally programmed coronary arteries.

O-016

Increased Inflammation and Fibrosis Aggravate the Course of Acute Mesangioproliferative Nephritis after Intrauterine Growth Retardation in the Rat

Christian Plank, Iris Östreicher, Andrea Hartner, Kerstin Amann, Wolfgang Rascher, Jörg Dötsch Department of Pediatrics University Erlangen-Nuremberg, Department of Pathology University Erlangen-Nuremberg, D-91054 Erlangen, Germany

Objective. Observational studies in children suggest that IUGR may be associated with a more severe course of kidney diseases such as IgA-nephropathy and nephrotic syndrome. We tested the hypothesis that IUGR aggravates renal disease during Anti-Thy 1.1. nephritis (GN), a self limiting acute mesangioproliferative glomerulonephritis, in rats. **Methods:** We induced IUGR in Wistar rats by isocaloric protein restriction (8% vs. 20%) in pregnant dams. Litter size was reduced to 6 male neonates in both groups, low protein animals (LP) and normal protein animals (NP). Mothers and weaned rats were fed standard rat chow. At the age of 10 weeks GN was induced by injection of an anti-Thy1.1.-antibody (ER4G, 1mg/kg body weight). Rats were sacrified 4 days and 14 days after induction of GN, respectively and organs were taken. Parameters of renal inflammation and fibrosis were measured by microscopy and real time PCR. **Results:** Excessive compensatory growth following IUGR led to a higher body weight in LP animals (336.8 ± 8 vs 313.5 ± 5 g in NP, $p<0.05$) and a higher mean arterial blood pressure (119 ± 4 in LP vs. 100 ± 13 in NP, $p<0.01$). GN LP animals showed a higher median glomerulosclerosis score than NP animals (1.86 (1.38- 1.98) vs. 1.2 (0.84-1.98), $p<0.05$) on day 14 which was due to a significantly higher percentage of severly damaged glomeruli (sores 3 and 4, 53±9 % in LP vs. 18±10% in NP, $p< 0.05$). In LP animals median interstitial lesion score was higher than in NP animals on day 4 (0.20 (0.10 - 0.45) vs 0.10 (0 - 0.10), $p< 0.05$) and on day 14 (1.10 (1.10 - 1.30) vs. (0.60 (0.10 - 1.25), $p=0.055$). LP animals tended to more extracapillary proliferates (7± 1 vs. 3 ± 2 % of the glomeruli in NP, $p= 0.112$). On day 14 there was a significant higher cortical mRNA expression in LP animals for TNF (10-fold, $p<0.05$), MCP-1 (19-fold, $p<0.05$), Osteopontin (2-fold, $p<0.05$), TGF-ß1 (2.5-fold, $p<0.05$) and collagen 1 (2.7-fold, $p<0.05$) compared to NP. **Conclusions:** We conclude that after IUGR increased inflammation and induction of fibrosis impair the reparation of the kidney after acute Anti-Thy-1.1.-nephritis, leading to more sclerotic lesions. Altered programming of kidney regeneration and reparation may be an underlying mechanism.

Parallel Session 2A: Lifecourse Data Analyses

O-017

Revisiting the Four–Model Principle in Fetal Origins Hypothesis

Yu-Kang Tu (1, 2), George TH Ellison (3), Mark S Gilthorpe (1); (1) Biostatistics Unit, Centre for Epidemiology & Biostatistics, LIGHT, University of Leeds, Leeds, LS2 9LN, UK; (2) Leeds Dental Institute, University of Leeds, Leeds, LS2 9LU, UK. (3) St George's, University of London, Cranmer Terrace, London SW17 0RE, UK

Background: In an influential study, Lucas et al. (1999) proposed a framework of regression analyses to test the fetal origins of adult disease hypothesis. They suggested that four regression models should be tested and reported by studies examining the relation between birth weight (BW) and health outcomes in later life. For example, if the outcome of interest were systolic blood pressure (SBP), Lucas et al. advised that results from the following four models should be tested and presented: Model 1 (early model): $SBP = a_1 + b_1BW$; Model 2 (later model): $SBP = a_2 + c_2CW$; Model 3 (combined model): $SBP = a_3 + b_3$ birth weight $+ c_3$ current body weight; and Model 4 (interaction model): $SBP = a_4 + b_4BW + c_4CW + d_4BW*CW$; where Lucas *et al.* claimed that b_1, b_3, b_4 and d_4 are expected to be negative under the fetal origins hypothesis. Certainly, from the literature, it is clear that b_1 tends to be negative, although only weakly so and is often very close to zero. Likewise, while b_3 is usually negative and statistically significant this is often due to inappropriate adjustment for CW, which produces the statistical phenomenon known as the "reversal paradox" (Tu et al., 2005). **Methods:** We examined the theoretical expected value of the partial regression coefficient for the product term (d_4) when the three continuous variables (SBP, BW, and CW) are multivariate normal. We then applied the four model analysis to data from 'Study 1' provided on the DOHaD 2005 Congress website. Finally, we conducted computer simulations to explore the impact of categorizing BW and/or CW before testing their interaction. **Results:** When the three continuous variables (SBP, BW, and CW) are multivariate normal, we were able to prove that the expected value of the partial regression coefficient for the product term (d_4) was zero, irrespective of covariate correlations. Analyses of the data from 'Study 1', confirmed that when 2-hour glucose concentration was used as the outcome, d_4 was very close to zero (-0.002 [95% confidence interval: -0.016, 0.012] for the interaction between BW and CW, and -0.003 [95% CI: -0.023, 0.018] for the interaction between BW and current body height). Finally, our computer simulations showed that dichotomizing BW and/or CW and then testing their interaction can give rise to spurious yet significant interaction coefficients, where d_4 can be both positive and negative. At the same time, the Type I error rate for d_4 is no longer 5%, but becomes inflated. Both the departure from zero and the inflated Type I error rates increase when there are stronger bivariate correlations between the original continuous covariates or when sample sizes are larger. **Conclusion:** These findings pose an interesting statistical conundrum that needs careful consideration when interpreting epidemiological evidence for the fetal origins hypothesis using Lucas et al.'s approach to catch-up growth, particularly since the interaction between size at birth (BW) and in later life (CW) is usually tested by categorizing BW and CW. **References:** Lucas A, Fewtrell MS, Cole TJ. Fetal origins of adult disease - the hypothesis revisited. BMJ 1999;319:245-249. Tu YK, West R, Ellison GTH, Gilthorpe MS. Why evidence for the fetal origins of adult disease might be a statistical artifact: the "reversal paradox" for the relation between birth weight and blood pressure in later life. Am J Epidemiol 2005;161:27-32.

O-018

Statistical Issues in Life Course Epidemiology

Bianca L. De Stavola, Dorothea Nitsch, Isabel dos Santos Silva, Valerie McCormack, Rebecca Hardy, Vera Mann, Tim J. Cole, Susan Morton, David A Leon. *Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine.*

There is growing recognition that the risk of many diseases in later life, such as type-2 diabetes or breast cancer, is affected by adult as well as early life variables, including those operating prior to conception and during the pre-natal period. Most of these risk factors are correlated because of common biological and/or social pathways, while some are intrinsically ordered over time. Their analysis raises several issues that are relevant to the current debate on causal inference in epidemiology. We give a brief overview of the main analytical and practical problems and consider alternative modelling approaches ranging from standard multiple regression (i.e. conditional) models to fully multivariate models, such as those used in path analysis and structural equation models. Issues arising from measurement error and missing data are also addressed. Examples originated from two UK cohorts are used to illustrate alternative modelling strategies. The first investigates how maternal and grand-maternal factors influence the size at birth of an offspring using the *Children of the 1950s Study*. This cohort includes all people who in 1962 participated in a reading survey while attending primary school in Aberdeen. The second example focuses on how adult leg length, which has been used in cancer and cardiovascular epidemiology as a marker of childhood environmental factors, is determined by different periods of childhood growth. Data from the *Medical Research Council National Survey of Health and Development* are used. It is concluded that more than one analytical approach should be adopted to gain more insight into the underlying mechanisms.

Parallel Session 2B: Neurologic and Mental Health Outcomes

O-019

Childhood Developmental Predictors of Mental Health During a Forty-Year Period from Adolescence to Adulthood

Ian Colman, Tim J. Croudace, George Ploubidis, Michael E.J. Wadsworth, Peter B. Jones. *Department of Psychiatry, University of Cambridge, Cambridge, United Kingdom; Medical Research Council National Survey of Health and Development, Department of Epidemiology and Public Health, University College London Medical School, London, United Kingdom.*

Background: Previous studies have suggested that early developmental factors can predict later mental health. These studies, however, have not considered mental health from a longitudinal perspective. The objective of this study was to identify developmental factors that would predict longitudinal phenotypes of mental disorder that covered a forty year period from age 13 to 53. **Methods**: Data from the Medical Research Council National Survey of Health and Development (the 1946 British birth cohort) was used. Survey members were assessed on seven occasions before age 11 for numerous factors related to development, including: developmental milestones such as age at first walking, height, weight, cognitive ability, academic achievement, alertness as assessed by a physician, speech defects, and presence of serious physical illness. Survey members (n=4,627) were assigned to one of six longitudinal phenotypes of mental disorder from age 13 to 53 using longitudinal latent class techniques: chronic mental disorder, adult onset mental disorder, adolescent mental disorder with positive mental health in adulthood, persistent minor difficulties, adult onset minor difficulties, and persistent positive mental health. Those with mental disorder and minor difficulties were compared to those with persistent positive mental health across the developmental factors. **Results**: Individuals with chronic mental disorder from age 13 to 53 significantly differed from those with persistent positive mental health across almost all aspects of development measured. Individuals with chronic mental disorder were more likely to sit up, stand, and walk for the first time at a later age (all $p<0.05$), have a shorter height at age 6 ($p<0.01$) and age 11 ($p<0.05$), have a lower body weight at age 6 ($p<0.01$) and age 11 ($p<0.05$), score poorly on cognitive tests at age 8 and age 11 (both $p<0.001$), be in the bottom quartile of their class in academic achievement at age 10 ($p<0.001$), to be rated as below average or apathetic with regards to alertness at ages 6, 7, and 11 (all $p<0.001$), to have speech defects at ages 6, 7, and 11 (all $p<0.05$), and to have a serious physical illness before age 11 ($p<0.001$). Individuals with adult onset disorder, disordered adolescents with positive adult mental health, and those with persistent minor difficulties fell between those with chronic disorder and those with persistent positive mental health across all of these developmental domains, though differences were rarely statistically significant. **Conclusions**: This study shows that numerous indicators of difficulties or delay during childhood development up to age 11 are significantly associated with chronic mental disorder from age 13 to 53. Furthermore, evidence from this study suggests that increased presence of

developmental difficulties across several domains is associated with increased severity and persistence of poor mental health across this forty year period.

O-020

Small Size at Birth is Associated with Indicators of Increased Hyperactivity, Reduced Attention and Reduced Effortful Control in Young Children Wolff Schlotz, David I. W. Phillips, Keith M. Godfrey, Alexander Jones; *MRC Epidemiology Resource Centre, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK.*

Background: Animal and human research suggests that a restrictive prenatal environment, reflected by small size at birth, may predict abnormal behaviour in later life. Much of the evidence for this in humans comes from studies of infants with abnormally low birthweight but little evidence is available over the normal birthweight range. Therefore, we have assessed temperament, emotion and behaviour in a group of normal, healthy prepubertal children using two parentally completed psychometric questionnaires. **Aim**: To assess how size at birth across a normal birthweight range is associated with later temperament, emotion, and behaviour in healthy young children. **Methods**: We have carried out a cross-sectional study of 68 boys and 72 girls (aged 7-9 years) who have been followed since 12 weeks of gestation when their mothers took part in a study of healthy children born in Southampton, United Kingdom. Whilst the children took part in a study of the impact of prenatal environment on physiological responses to psychological stress, the mothers completed a Strengths and Difficulties Questionnaire (SDQ) and a short form of the Child Behaviour Questionnaire (CBQ) about their child and a General Health Questionnaire (GHQ) about themselves. **Results**: The median total difficulties score from the SDQ was 9 (IQR 7.5). Risk of psychosocial difficulties is considered to be increased for scores above 13. Total difficulties scores were greater in children who had lower weight ($r = -.21$, $P = 0.01$), smaller head circumference ($r = -.20$, $P = 0.01$) and were shorter ($r = -.17$, $P = 0.04$) at birth. Of the subscales of the SDQ, scores on the hyperactivity scale, which also assesses inattention and impulsivity, were significantly greater in children who had lower weight ($r = -.21$, $P = 0.008$), smaller head circumference ($r = -.23$, $P = 0.005$) and were shorter ($r = -.21$, $P = 0.01$) at birth. No associations between size at birth and the emotional symptoms, conduct problems, peer problems, or prosocial behaviour scales were found. Of the scales of the CBQ that we assessed, negative affect and surgency were not associated with size at birth whilst effortful control was significantly less in children who had lower weight ($r = .15$, $P < 0.05$) and smaller head circumference ($r = 0.21$, $P = 0.006$) at birth. Effortful control describes socialised conduct where an individual is able to suppress a strong behavioural urge in favour of a more socially acceptable response to a situation or demand. As maternal smoking during pregnancy, social class and the child's gender all had significant effects on our outcome measures, we adjusted for their confounding influence in our analysis. Our findings were also independent of gestational age, maternal alcohol intake during pregnancy, and maternal anxiety or depression as assessed by the GHQ. **Conclusion**: This study demonstrates significant relationships between markers of fetal growth restriction and indicators of hyperactivity, inattention and reduced effortful control in children. Previous case-control studies of children with Attention Deficit Hyperactivity Disorder (ADHD) show a significantly greater risk of this condition at the extremes of low birthweight. However, little data exists about the effect of birthweight on features of ADHD over the normal range. Our findings suggest that measures of temperament and behaviour, similar to symptoms of ADHD, are increasingly likely as birthweight falls across the normal range.

O-021

The Effect of Breast Feeding on Vision and Myopia in Children and Young Adolescents Christopher G Owen, Alicja R Rudnicka, David P Strachan; Division of Community Health Sciences, St George's, University of London, Cranmer Terrace, London, UK, SW17 0RE.

Background: Myopia represents a major public health problem in both developed and developing countries, which has increased in prevalence in the past 2 decades or so, especially amongst industrialised societies. It has recently been suggested that infant feeding is associated with myopia in childhood. We examine the influence of infant feeding on vision, visual acuity and myopia in childhood and adolescence. **Methods:** Two British Cohort Studies recruited 18,558 babies born in one week in March 1958 and 16,567 born in one week in April 1970. Participants were followed up at different ages through childhood. A parental questionnaire ascertained infant feeding status at 5 or 7 years of age. Unaided vision and corrected visual acuity were measured at 11 and 16 years in the 1958 cohort, and 10 and 16 years in the 1970 cohort using Snellen charts. Odds ratios of vision 6/12 or worse (equivalent to a LogMAR acuity 0.3 or more), vision 6/12 or worse and near vision of 6/6 or 6/9 at 25 cm (equivalent to approximately -1.00 to -5.00 dioptres of myopia), and best corrected visual acuity 6/12 or worse (vision used if corrected acuity was not recorded; better eye vision or visual acuity is used throughout), among subjects breast fed for 1 month or more compared with formula fed subjects. Odds ratios less than one imply a beneficial effect of breast feeding on visual outcome. Odds ratios are adjusted for socioeconomic status, parental education, maternal age at delivery, child's birth weight and birth order throughout. **Results:** At 11 years of age in the 1958 cohort, 6.8% of children (n=584/8596) had vision 6/12 or worse, 5.5% were likely to be myopic and 2.8% had a best eye corrected acuity or vision of 6/12 or worse; figures for the 1970 cohort at age 10 are 4.5% (n=401/9012), 3.5% and 2.1% respectively. Effects of infant feeding on childhood visual outcome are summarised in the table.

Vision Outcome	Prevalence: breast fed vs. formula fed, p-value (Adjusted odds ratios, 95% confidence intervals, p-value)	
	1958 cohort age 11 yrs	1970 cohort age 10 yrs
Distance vision 6/12 or worse	7.0% vs 6.5%, 0.42 (1.02, 0.84 to 1.23, 0.85)	5.5% vs. 4.1%, 0.012 (1.17, 0.92 to 1.48, 0.19)
Suspected myopia	5.9% vs. 5.0%, 0.06 (1.09, 0.88 to 1.35, 0.41)	4.6% vs. 3.1%, 0.001 (1.26, 0.96 to 1.63, 0.09)
Best attainable visual acuity 6/12 or worse	2.8% vs. 3.7%, 0.024 (0.79, 0.61 to 1.03, 0.08)	2.4% vs. 2.0%, 0.29 (1.18, 0.84 to 1.66, 0.33)

At 16 years the prevalence of myopia was marginally higher in those breast fed in the 1958 cohort only (8.4% vs. 7.1%, p=0.046); this effect was attenuated after adjustment (1.15, 95% CI 0.93 to 1.41). No association between infant feeding status, vision, and visual acuity at 16 years was observed with or without adjustment in either cohort. **Conclusions:** Recent findings of a protective effect of breast feeding on myopia in Asian children are not supported in these large prospective studies of UK children. The apparent beneficial effect of formula feeding over breast feeding on visual outcome is explained by confounding, and infers that familial and social patterning of reduced vision and myopia account for the association between infant feeding and visual outcome in later life.

O-022

Small Body Size at Birth in Term Infants Predicts Behavioural Symptoms of ADHD in Preschoolers Lahti, J.[1], Räikkönen, K.[1], Kajantie, E.[2], Pesonen, A.[1], Heinonen, K.[1], Järvenpää, A-L.[3], Strandberg, T.[4] 1: Department of Psychology, University of Helsinki, Helsinki, Finland, 2: National Public Health Institute, and Hospital for Children and Adolescents, Helsinki University Central Hospital, 3: Hospital for Children and Adolescents, Helsinki University Central Hospital, Department of Neonatology. 4: Department of Medicine, University of Helsinki.

Background: Behavioural disorders with neurodevelopmental background, such as attention deficit hyperactivity disorder (ADHD), has been associated with developmental environment *in utero*. Majority of the studies have focused on extremely low end of the distribution in birth size, and have shown that infants born either low weight or small for gestational age may have increased risk for developing behavioural problems in general and attention deficits in particular. However, whether a similar association exists among the normal range of term births is poorly known. **Methods**: A sample of 267 mother-father-infant triads were studied to explore whether body size at birth predicts parent-reported behavioural symptoms of ADHD measured with standardized questionnaire at five to six years among infants born healthy at term. Person-centered approach was applied to test the hypothesis that infants born small would cluster together and the cluster membership would associate with later behavioural symptoms of ADHD. **Results**: Behavioural symptoms of ADHD at five-year-old children were predicted by small body size at birth, characterized as low ponderal index, small head circumference, and head circumference-to-height –ratio index (β's : - 0.12 to -0.14, p's < .05), and the associations remained significant even after adjustment for gestation age, maternal tobacco use during pregnancy, socio-economic status of the family, maternal BMI, mother's age, and gender of the child. Further analysis indicated that cluster of infants characterized with small body size at birth had 1.9 (CI = 1.4 – 3.0) times greater risk for belonging to the cluster with high score on mothers' and fathers' ADHD ratings. **Conclusion**: The results suggest that mechanisms that link small body size at birth with preschool age behavioural symptoms of ADHD may be operative not only in extreme groups but also within the normal range of term births.

Parallel Session 2C: Placenta

O-023

Impact of Superovulation, Artificial Insemination, Embryo Transfer, and in vitro Culture on Fetoplacental Growth in the Sheep in Late Gestation Severence M MacLaughlin[1], Simon K Walker[2] and I Caroline McMillen[1]; [1]Discipline of Physiology, The School of Molecular and Biomedical Science, University of Adelaide, [2]South Australian Research and Development Institute. Adelaide, South Australia, Australia.

Introduction: Superovulation, artificial insemination, embryo transfer and in vitro embryo culture are used in a range of assisted reproductive technologies and it has been demonstrated that varying the composition of the culture medium can result in a change in pre and postnatal development. Culture of sheep embryos in media containing serum is associated with fetal overgrowth. It is not known how the combination of superovulation, artificial insemination and embryo transfer alone impacts on fetoplacental development in late gestation. There have been no studies, however, examining the differential impact of superovulation, artificial insemination and embryo transfer alone or combined with IVC on singleton and twin pregnancies. **Objective:** To test the hypothesis that IVC in a medium containing human serum results in an increase in fetal and placental weights and that there is a differential effect in singleton and twin pregnancies. **Methods:** Embryos were collected 24h after artificial insemination of superovulated donor ewes, which were subsequently transferred to one of 3 treatment groups: - to intermediate ewes until day 7 [Embryo transfer, ET group (singletons = 6, Twins = 18)]; -to an in vitro culture of synthetic oviductal fluid either without [ET+IVC+No Serum(NS) (singletons = 7, Twins = 23)] or with human serum [ET+IVC+Human Serum (HS) (singleton = 8, Twins = 4)] until day 6. Embryos were then transferred to final recipient ewes. In addition, naturally mated (NM) ewes were used as controls in this experiment [singletons = 9, twins = 15].

At 144/145d gestation, ewes were killed, and fetoplacental parameters were measured. **Results:** There was a decrease ($P < 0.01$) in both singleton and twin pregnancies in the weight of A type placentomes in the ET (28.4±9.1g), ET+IVC+NS (27.1±12.4g), and ET+IVC+HS (31.7±11.8g) groups compared to the NM (129.5±40.6g) group. Amniotic fluid was significantly increased ($P < 0.05$) in the ET+IVS+HS. In singleton pregnancies, fetal weight was higher ($P < 0.03$) in ET+IVC+HS (5.8±0.1kg) when compared to the ET (4.8±0.1kg), ET+IVC+NS (5.0±0.1kg), and NM (4.6±0.1kg) groups. Interestingly, in twin pregnancies there was a significant decrease ($P < 0.01$) in fetal, placental, and mean placentome weights respectively in the ET (3.9±0.1kg, 327±10g, and 9.4±0.3g), ET+IVC+NS (4.1±0.1kg, 343±7g, and 8.4±0.2g) and ET+IVC+HS (4.8±0.1kg, 367±30g, and 8.3±0.8g) groups compared to the NM (5.2±0.1kg, 518±14g, and 13.6±0.4g) group. **Conclusion:** We have demonstrated that the use of superovulation, artificial insemination, and embryo transfer alters placental development in late gestation. Furthermore, embryo transfer and in vitro culture have a differential impact on fetal and placental growth in singleton and twin pregnancies.

O-024

Gestational Changes in Placental Function in Rats Fed a Low Protein Diet

Nina Jansson, Jessica Pettersson, Anette Ericsson, Isabelle Palmberg, Theresa. L. Powelland Thomas Jansson. Perinatal Center, Department of Physiology and Pharmacology, Göteborg University, Sweden

Background: Altered maternal nutrition is proposed to represent one important factor underlying fetal programming. Indeed, offspring of rat dams given low protein diet during pregnancy have been shown to develop increased blood pressure and diabetes in adult age. However, the mechanisms causing restricted fetal growth and programming of fetal physiology during maternal protein restriction are unclear. We tested the hypothesis that the placenta down-regulates transport systems for amino acids in response to maternal low protein diet and, as a consequence, restricts fetal growth. To this effect, we studied the time course of changes in placental transport functions and fetal/placental growth in pregnant rats subjected to protein malnutrition. **Methods:** Pregnant Sprague Dawley rats were given a low protein diet (LP, 4% protein) or a control diet (C, 18% protein). The diets were isocaloric and introduced at gestational day (GD) 2. The rats were studied at either GD 15 (n= 26), GD 18 (n= 44), GD 19 (n= 27) or GD 21 (n= 28) (term 23 days). At each gestational age animals were further divided into two subgroups: In subgroup 1 animals were sacrificed, placentas and fetuses were removed and weighed and the protein expression of System A and mTOR was analysed by Western blot in pooled placental homogenates. Animals in the second subgroup were anesthetised 3 days prior to the day of experiment and catheters were inserted into the right jugular vein and right carotid artery. Transplacental transport studies were carried out by intravenous administration of isotope labelled methyl-glucose and MeAIB in awake animals. Maternal plasma insulin and leptin were analysed by RIA. **Results:** There were no changes in fetal and placental weights at GD 15, 18 or 19 in the low protein group compared to controls. However both fetal and placental weights were significantly decreased ($p<0.05$) at GD 21 in the LP group (FW: 2.76 g ± 0.12; PW 0.42 g ± 0.014) compared to the control group (FW: 3.5 g ± 0.061; PW: 0.48 g ± 0.013). The dams that were given control diet gained significantly ($p<0.05$) more weight during pregnancy from GD 18 in the catheterized group and from GD 19 in the non-catheterized group than the LP group did. Protein expression of System A (SNAT 2) was significantly reduced by 40% at GD 21 in the LP group compared to the control group. Preliminary data shows a decrease in mTOR expression at GD 18 in the LP group. There were no significant differences in placental uptake and transport of methyl-glucose between the control and LP groups. In contrast, placental uptake and transport capacity of MeAIB was significantly reduced in the LP group at GD 19 and 21. Preliminary data indicates that maternal plasma concentrations of both leptin and insulin were significantly ($p<0.05$) decreased in the LP group. **Discussion:** These data provide evidence that changes in placental nutrient transport function (GD 19) precede the development of fetal growth restriction (GD 21). The placenta responds to maternal malnutrition by altering functions such as amino acid transport, compatible with the suggestion that the placenta functions as a "nutrient sensor" We have previously shown that insulin and leptin upregulate system A in human placental fragments. Therefore, decreased levels of insulin and leptin as a metabolic response to maternal protein restriction may represent a mechanism for the decreased placental system A activity seen in the LP group. In conclusion, these data suggest that the altered fetal growth in response to maternal protein restriction is mediated through changes in placental nutrient transporters. Thus, alterations in placental transport functions may play an important role in fetal programming.

O-025

Preventing Maternal Growth in the Pregnant Adolescent Dam is Associated with Altered Placental Vascularity and Modest Fetal Growth Restriction

J.S. Luther[1,2], J.S. Milne[1], R.P. Aitken[1], M. Matsuzaki[1], D.A. Redmer[2], L.P. Reynolds[2] and J.M. Wallace[1]. [1]Rowett Research Institute, Aberdeen, AB21 9SB, UK. [2]North Dakota State University, Fargo, North Dakota, 58105, USA.

Background: The risks of miscarriage, prematurity and low birth weight are particularly acute in young adolescent girls who are still growing at the time of conception. As many girls who become pregnant have inadequate or marginal nutritional status during gestation, we have developed a sheep model to examine the effect of limiting maternal nutrient intake on conceptus growth. The present study focuses on placental growth and vascular development in these undernourished dams. **Methods**: Singleton pregnancies to a single sire were established by embryo transfer in adolescent ewes (~ 7 months old, adiposity score 2.3 units). Thereafter, dams were offered a moderate (M) or low (0.7 x M) level of a complete diet to maintain or deplete maternal adiposity, respectively. Bromo-deoxyuridine (BrdU) was administered 1 hour before slaughter on either day 90 or 130 of gestation. Placentomes were either (i) snap frozen for extraction of total cellular RNA and measurement of angiogenic factor gene expression, (ii) immersion fixed with Carnoys solution and BrdU labelled nuclei determined by immunohistochemistry or (iii) perfusion fixed with Carnoys followed by a vascular casting resin via the main vessel supplying the maternal caruncle (CAR) or fetal cotyledon (COT). Perfused tissues were stained with haematoxylin and periodic acid-Shiffs and vascular parameters evaluated by image analysis. **Results**: Maternal adiposity was maintained in moderate intake adolescent dams throughout, while adiposity scores steadily decreased in the underfed dams (Table). Circulating glucose concentrations and fetal weight were independent of maternal intake at day 90, but by day 130 both maternal and fetal glucose concentrations were suppressed in the low intake group. This was associated with a modest reduction ($P<0.01$) in fetal weight which was independent of changes in placental mass or cellular proliferation. Placental mRNA expression for 5 angiogenic ligands or receptors was lower ($P<0.001$) at day 90 versus day 130, but largely unaffected by maternal intake. In contrast, key indices of placental vascularity were attenuated in the underfed group at both stages.

Table. Selected placental parameters in relation to fetal growth in undernourished adolescents, n=7 per group

	Day 90 of gestation		Day 130 of gestation	
	Moderate	Low	Moderate	Low
Maternal adiposity (score units)	2.32 ± 0.046	1.89 ± 0.051***	2.36 ± 0.051	1.61 ± 0.051***
Maternal glucose (μmol/ml)	3.2 ± 0.13	3.0 ± 0.06	3.3 ± 0.10	2.7 ± 0.06**
Placentome mass (g)	664 ± 75.6	607 ± 28.0	480 ± 28.7	469 ± 39.8
Placentome proliferation index (%)	9.3 ± 0.48	9.0 ± 0.36	4.9 ± 0.30	5.1 ± 0.30
COT vessel area density (%)	10.5 ± 0.70	12.3 ± 0.86	21.3 ± 1.02	20.8 ± 1.67
COT vessel surface density ($\mu m/\mu m^2$)	4.1 ± 0.20	5.0 ± 0.45*	7.7 ± 0.48	8.4 ± 0.63
CAR vessel area density (%)	41.7 ± 1.73	34.1 ± 0.70**	59.2 ± 1.23	47.6 ± 2.64**
CAR vessel surface density ($\mu m/\mu m^2$)	9.5 ± 0.31	8.4 ± 0.16**	9.7 ± 0.25	9.5 ± 0.53
Fetal glucose (μmol/ml)	0.49 ± 0.038	0.47 ± 0.055	0.40 ± 0.076	0.22 ± 0.030*
Fetal mass (g)	657 ± 30.0	597 ± 42.7	4273 ± 83.8	3555 ± 152.2**

Within day of gestation *P<0.05, **P<0.01, ***P<0.001

Conclusion: Undernourishing the pregnant adolescent dam decreases fetal nutrient supply and restricts fetal growth independent of changes in placental mass. Alterations in placental vascular development may play a role in mediating the reduction in fetal nutrient supply. *Funded by SEERAD and NIH grant HD45784*

O-026

Adaptation of Nutrient Supply to Fetal Demand in the Mouse Involves Interaction Between the *Igf2* Gene and Placental Transporter Systems

I. Sandovici[1], E. Angiolini[1], P. Smith[1], R. J. Smith[1], G. Kelsey[1], W. Dean[1], A. C. Ferguson-Smith[2], C. P. Sibley[3], W. Reik[1], A. L. Fowden[4] and M. Constância[1] [1]Laboratory of Developmental Genetics and Imprinting, The Babraham Institute, UK [2]Department of Anatomy, University of Cambridge, UK; [3]Division of Human Development, Medical School, University of Manchester, UK; [4]Department of Physiology, University of Cambridge, UK.

The mammalian fetus is unique in its dependence during gestation on the supply of maternal nutrients through the placenta. Maternal supply and fetal demand for nutrients need to be fine tuned for healthy growth and development of the fetus along its genetic trajectory; an altered balance between supply and demand can lead to deviations from this trajectory with long term consequences for health. We have previously shown that in a knockout lacking the imprinted placental-specific *Igf2* transcript (P0), growth of the placenta is compromised at E16 but fetal growth is still normal, suggesting functional adaptation of the placenta to meet the fetal demands. Here we show that placental transport of glucose and amino acids are increased in the *Igf2* $P0^{+/-}$ null and that this up-regulation of transport occurs through increased expression of the transporter genes *Slc2a3* and *Slc38a4*, the imprinted member of the System A amino acid transporter gene family. Decreasing fetal demand genetically by removal of fetal *Igf2* abolished up-regulation of both transport systems at E16 and reduced placental System A amino acid transport activity and expression of *Slc38a2* in late gestation. Our results provide the first direct evidence that the placenta can respond to fetal demand signals through regulation of expression of specific placental transport systems. Thus crosstalk between an imprinted growth demand gene (*Igf2*) and placental supply transporter genes (*Slc38a4, Slc38a2, Slc2a3*) may be a component of the genetic control of nutrient supply and demand during mammalian development. We are currently dissecting the mechanisms involved in up-regulation of *Slc38a4* and *Slc2a3* expression in *Igf2* $P0^{+/-}$ null mice.

Parallel Session 2D: Clinical and Public Health Interventions

O-027

BMI Charts for Screening for Childhood Growth Associated with Adult Metabolic Disease Harshpal S. Sachdev, Clive Osmond, Caroline H.D. Fall, Ramakrishnan Lakshmy, Sushant K. Dey Biswas, Dorairaj Prabhakaran, Kolli Srinath Reddy, David J.P. Barker, and Santosh K. Bhargava, *Department of Pediatrics, Maulana Azad Medical College, New Delhi 110002, India, Medical Research Council Epidemiology Resource Centre, University of Southampton, Southampton General Hospital, Southampton SO16 6YD, UK, Department of Cardiology, All India Institute of Medical Sciences, New Delhi 110029, India, Indian Council of Medical Research, New Delhi 110029, India, and Department of Pediatrics, Sunder Lal Jain Hospital, Delhi 110052, India.*

Background: Sustained accelerated childhood Body Mass Index (BMI) gain (crossing centiles upwards) is associated with adult metabolic disorders. Current preventive efforts are primarily focused on obese or overweight children, identified from cross-sectional, 'distance' BMI charts. This strategy, often based on international definitions, ignores relative changes in childhood BMI, and is sub-optimal for developing countries undergoing nutrition transition. For example, young adult Indians with impaired glucose tolerance (IGT) or diabetes were not overweight or obese as children but were characterized by their high rate of gain in body mass. **Objective:** To develop BMI charts for routine use in children to recognize growth associated with adult metabolic disease. **Methods:** We evaluated anthropometry, serum biochemistry, and plasma insulin concentrations in 1492 men and women 26 to 32 years of age who had been measured at birth and at intervals of 3 to 6 months throughout infancy, childhood, and adolescence in a prospective, population-based study (New Delhi birth cohort). Age and sex specific BMI charts were constructed from the cohort data for screening for adult outcomes of metabolic syndrome, and IGT or diabetes. **Results:** Point estimates of BMI categories (2/3 SD bands) at ages 4 up to 12 years were positively related to adult metabolic syndrome (adjusted ORs 1.20 to 1.77; P 0.006 to < 0.001). Point estimates of BMI only at 12 years were positively related to adult IGT or diabetes (OR 1.24; P 0.003). Relative changes (gain) in BMI in 3-year intervals commencing from the age of 5 years (5-8, 6-9, 7-10, 8-11, and 9-12 year intervals) predicted adult metabolic syndrome (adjusted ORs 1.34 to 1.71; P ≤ 0.001), and IGT or diabetes (ORs 1.23 to 1.97; P 0.06 to < 0.001). Conjoint use of two types of color-coded BMI charts, based on risk of adult morbidity, emerged as a feasible screening option: (i) an inverse graph directly providing the SD scores from the observed BMI values between 5 and 12 years of age, and (ii) charts relating the BMI SD values between 8 and 12 years of age to the change in BMI SD scores in the preceding 3 year interval.
Conclusion: It is feasible to develop BMI charts for routine screening for childhood growth associated with adult metabolic disease.

O-028

IVF Children are Taller with Increased IGF-I, IGF-II and IGFBP-3 Levels Suggesting Altered Genetic Imprinting Harriet Miles, Paul L Hofman, Wayne S Cutfield. *Liggins Institute, University of Auckland, Auckland, New Zealand*

Culture of pre-implantation mammalian embryos can affect the methylation and therefore expression of imprinted genes involved in growth regulation including IGF-II. In mice the imprinting of genes such as RASGRF1 alter the growth hormone-IGF axis and body size. Changes in genomic imprinting have also been shown to alter lipid and energy metabolism. The long-term outcome of children conceived by IVF is unknown however a nine-fold increase in the imprinted gene disorder Beckwith Wiedemann Syndrome has been reported. **Hypothesis:** IVF results in altered methylation of imprinted genes involved in growth and metabolism leading to measurable changes in the phenotype and hormonal profile in mid-childhood. **Methods:** Healthy, pre-pubertal children born at term following singleton pregnancy were evaluated. Subjects comprised an IVF group conceived from fresh embryo transfer and a naturally conceived control group. Anthropometric measurements, bone age, DEXA scan (Lunar prodigy 2000), fasting plasma glucose, insulin, lipid profile, IGF I, IGF II, IGFBP3 and GHBP were performed on all subjects. Values expressed as mean ±SEM. **Results:** There were 51 IVF (6.0±0.2 yrs, 23 males) and 56 control subjects (7.0±2.0 years, 26 males). IVF subjects were taller than controls when corrected for mid-parental height (MPH; height SDS–MPHSDS 0.37±0.1 versus –0.30±0.1, p<0.0001), with a larger difference seen in girls (height SDS-MPH SDS 0.62 ± 0.98 versus –0.33 ±0.88 p<0.005) than boys (Ht SDS–MPH SDS 0.62±0.98 versus 0.028 ±1.0, p=0.03). IVF subjects were leaner than controls (BMI SDS p=0.049), which was more apparent in IVF boys (BMI SDS –0.08±0.11 versus 0.72±0.15, p=0.02). IVF children had higher values for IGF-I (104±5 versus 80±4 ng/ml, p=0.003), IGF-II (78±2 versus 67±2 ng/ml, p<0.0001) and IGFBP-3 (4.85±0.09 versus 4.51±0.08 ng/ml, p=0.009). IVF children had a more favourable lipid profile with lower HDL:cholesterol ratio (2.6±0.08 versus 2.9±0.07 mmol/l p=0.03) and lower triglyceride levels (0.63±0.03 versus 0.75±0.03, p=0.003). **Conclusions:** IVF children are taller and leaner with higher plasma IGF-I, IGF-II, IGFBP-3 levels and a more favourable lipid profile. We speculate that in vitro periconceptual manipulation has altered genetic imprinting in growth and lipid regulating genes in IVF children.

O-029

How Effectively do Young Women Prepare for Pregnancy? Hazel M. Inskip, Sarah R. Crozier, Sharon E. Borland, Siân M. Robinson, Keith M. Godfrey & the Southampton Women's Survey Study Group. *MRC Epidemiology Resource Centre, University of Southampton, Southampton SO16 6YD*, UK.

Background. Women in Britain who are planning a pregnancy are advised not to smoke, to drink no more than one to two units of alcohol one to two times a week, and to take 400 µg of folic acid supplements each day[1]. Little is known, however, about how closely women who are planning a pregnancy follow the guidelines. Using data from the Southampton Women's Survey that were collected when the women were not pregnant, we have been able to examine this issue. Methods. We interviewed over 12,500 non-pregnant Southampton women aged 20-34 years for the Southampton Women's Survey. The women were asked whether they currently smoked, how much alcohol they consumed, and details of all dietary supplements taken (from which the intake of folic acid from supplements was derived). Data are available for the first 6,083 women who were interviewed between April 1998 and June 2000. The sample of women is representative of the British population in terms of ethnicity, and has a similar range of Townsend index scores (a measure of deprivation). We compared women who became pregnant within three months of interview with those who did not, and subdivided each of these groups according to whether the woman had said at interview that she anticipated trying for a baby within the next year. Results. Among the 102 women who became pregnant within three months of the interview, 12 (12%) were taking the recommended dose of folic acid, 73 (72%) did not smoke, and 53 (52%) of them drank on average no more than four units of alcohol per week; 10 (10%) were taking the recommended amount of folic acid as well as refraining from smoking, and drinking no more than four units of alcohol per week. The table shows that only 12% of women who anticipated trying for a baby and who conceived within three months followed all the guidelines in relation to folic acid, smoking and alcohol consumption. Women in this group were, however, marginally more likely to limit their alcohol consumption and to be non-smokers than women in the other groups.

	Not pregnant within 3 months		Pregnant within 3 months	
	Not trying n=5086	Trying for baby n=895	Not trying n=24	Trying for baby n=78
Folic acid ≥ 400 µg/day	48 (1%)	68 (8%)	1 (4%)	11 (14%)
Does not smoke	3335 (66%)	638 (71%)	15 (63%)	58 (74%)
≤4 units alcohol/week	2243 (44%)	436 (49%)	12 (50%)	41 (53%)
Non-smoker & ≤4 units alcohol/ week & ≥400 µg folic acid /day	26 (1%)	47 (5%)	1 (4%)	9 (12%)

Conclusion. Although some women who are planning pregnancies achieve the recommendation to take ≥400 µg/day of folic acid prior to conception as well as refraining from smoking, and drinking no more than four units of alcohol per week, they are very much in the minority. Even in women planning a pregnancy, around 90% do not follow current health guidelines.

[1] http://www.eatwell.gov.uk/agesandstages/pregnancy/trybaby/

Parallel Session 3A: Maternal Nutrition

O-030

Impaired Insulin Secretion after Prenatal Exposure to the Dutch Famine Susanne R. de Rooij[1], Rebecca C. Painter[1], Tessa J. Roseboom[1], David I.W. Phillips[2], Clive Osmond[2], David J.P. Barker[3], Michael W. Tanck[1], Robert P.J. Michels[4], Ian F. Godsland[5], Patrick M.M. Bossuyt[1] and Otto P. Bleker[6]; [1]*Department of Clinical Epidemiology and Biostatistics, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands,* [2]*MRC Epidemiology Resource Centre at the University of Southampton, Southampton, UK,* [3]*Developmental Origins of Adult Disease Centre, University of Southampton, Southampton, UK,* [4]*Department of Internal Medicine, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands,* [5]*Wynn Department of Metabolic Medicine, Imperial College School of Medicine, London, UK,* [6]*Department of Obstetrics and Gynaecology, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands*

Background Restricted early growth is associated with a higher prevalence of impaired glucose tolerance and type 2 diabetes in later life. This association is hypothesized to originate through adaptations made by the fetus when nutrient supply fails to meet demand. There is accumulating evidence that restricted early growth leads to insulin resistance. Previously we found that prenatal exposure to the Dutch famine is linked to impaired glucose tolerance, but the mechanisms are not known. **Aim** To determine whether the association between exposure to famine in utero and decreased glucose tolerance is mediated through alterations in insulin secretion, insulin sensitivity or a combination of both. Methods We performed a fifteen point intravenous glucose tolerance test in a sub-sample of 100 normoglycaemic men and women from the Dutch Famine birth cohort. This cohort consists of term singletons born around the time of the 1944-1945 Dutch famine. We used the minimal model of glucose disappearance to analyse the data. In all analyses we adjusted for sex, age and body mass index. Results Overall glucose tolerance was impaired in people who had been exposed to famine during mid gestation (difference in overall glucose tolerance index $=3.7*10^{-2}\cdot min^{-1}$ (95% CI : 0.2 to 6.5)) and early gestation (difference=$5.4*10^{-2}\cdot min^{-1}$ (95% CI: 2.2 to

8.0)) compared to people unexposed to famine in utero. People exposed to famine during mid gestation had a lower first phase insulin response to glucose (AIR_{Glc}) (-82 $min \cdot pmol \cdot l^{-1}$ (95% CI : -144 to 4)) and a lower disposition index (DI) (-66 (95% CI: -106 to -8)) compared to people unexposed to famine. DI, derived as the product of insulin sensitivity (Si) and AIR_{Glc}, is a measure of the activity of the B-cells adjusted for insulin resistance. Prenatal exposure to famine during early gestation also seemed associated with lower levels of AIR_{Glc} and DI, but these differences did not reach statistical significance. **Conclusions** The impaired glucose tolerance after exposure to famine during mid and early gestation seems to be mediated through an insulin secretion *defect.*

O-031

Does the Maternal Micronutrient Deficiency (Cu or Zn or Vit E) Modulate the Expression of Placental 11 ? Hydroxysteroid Dehydrogenase and *Per Se* Predispose the Offspring to Insulin Resistance and Hypertension in Later Life?

Rosario JF[1], Anbu Gomez P[3], Patrick Gomez M[2]. FOAD Unit, Department of [1]Biochemistry and [2]Biotechnology, St.Joseph's College (Autonomous), Tiruchirappalli-620 002, [3]Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, INDIA.

Background: Epidemiological evidence suggests that some adult disorders like insulin resistance syndrome, hypertension and disease associated with it originate in fetal life. Maternal under nutrition is hypothesized to predispose the offspring to disease in adult life. The relevance of maternal macronutrient deficiency has been well studied but not that of micronutrients. We hypothesize that chronic maternal dietary mineral (Cu or Zn or Fe) or vitamin E restriction modulate the expression of placental 11 ß Hydroxysteroid Dehydrogenase-2 (11 ß HSD-2) *per se* individually predisposes the offspring to insulin resistance syndrome and hypertension in their later life. **Objective**: To assess the effect of maternal dietary mineral (Cu or Zn or Fe) or vitamin E restriction *per se* on intraperitoneal glucose tolerance test (IPGTT), insulin resistance (IR) and blood pressure in offspring and to study their effects on placental expression profile of 11 ß Hydroxysteroid Dehydrogenase-2 **Methods:** Female weaning Swiss albino mice received a control diet (based on the American Institute of Nutrition AIN-93G diet) (n= 20) or a 50% vitamin E-restricted diet (n=20) or 50% zinc restricted diet (n=20) or 50% copper restricted diet (n=20) or 50 % of iron restricted diet (n=20) and they were provided with deionized water for 12 weeks and mated with control males. Pups born to the dams on the restricted diet were weaned on to the respective restricted diet. At the end of 20 weeks of feeding of the offspring, IPGTT was performed in the offspring. After overnight fasting, glucose at a dose of 2.0 g/kg body weight was administered intraperitoneally and blood samples were obtained from the orbital sinus at 0, 60 and 120 min for determination of plasma glucose and insulin concentrations. Total cholesterol and triglycerols were measured in fasting plasma using commercially available Kits. Systolic blood pressure was determined at 20 weeks-old offspring by tail cuff plethysmography using an IITC model-229 blood pressure monitor. In 50 % of animals, pregnancies (n=10/ each diet group) were terminated on day 20 of gestation. Placentas and fetuses were excised and weighed. **Molecular analysis**: Placenta samples were used for western blot and starch gel electrophoresis analysis of 11 ß Hydroxysteroid Dehydrogenase-2 in both control and micronutrient deficiency groups. **Results**: Pregnant micro nutrient restricted dams had a higher abortion rate. Body weight and crown rump length were significantly ($p<0.001$) reduced in offspring, from dams fed micronutrient diet compared with control group. The micro nutrient restricted (Cu or Zn or Fe or Vit E) mice had significantly impaired glucose tolerance compared with control group. Glucose intolerance in association with hyperinsulinemeia and increased systolic blood pressure suggests the presence of insulin resistance and hypertension in all the offspring of micro nutrient restricted groups. Fasting plasma total cholesterol and triglycerides were higher in offspring of micronutrient restricted group. Western and starch gel electrophoresis analysis (as shown in Fig-1) of 11 ß HSD-2 expressions in placenta were down regulated in all the restricted diet fed groups. **Conclusion:** It has been suggested that chronic maternal micro nutrient deficiency alters11 ß HSD-2 expression and predispose the offspring to IR and hypertension in later life

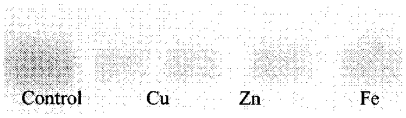

Fig.1.Western blot analysis of 11 ß HSD-2 expression in protein isolated from the placenta of mice fed with control Vs micronutrient restricted diet group.

O-032

Protein and Calcium Intake of Pregnant Women and Blood Pressure of their Children Vivienne Moore, Michael Davies, Kristyn Willson, Tony Worsley, Jeffrey Robinson; Department of Public Health, University of Adelaide; Research Centre for Reproductive Health, Department of Obstetrics & Gynaecology, University of Adelaide; School of Health Sciences, Deakin University, Australia.

Background A small number of published studies suggest that the protein content of a woman's diet during pregnancy, and supplemental calcium, may influence the blood pressure of her child. Blood pressure values of children convey information about the later risk of cardiovascular disease as blood pressure exhibits "tracking" over the life-span. Methods Blood pressure of 442 children was assessed at 3½ years of age, using an automated oscillometric recorder. The children were part of a birth cohort of 557, with families broadly representative of those in the wider community. At the time of follow up, 544 families were still part of the cohort (2 deaths, 11 withdrawals), and some information was obtained from 511 families (92%). For 230 girls, mean weight and height were 16 kg (sd 2 kg) and 99 cm (sd 4 cm), respectively. The 212 boys were, on average, 1 kg heaver and 2 cm taller. Differences in weight accounted for blood pressure differences between boys and girls. Mean systolic blood pressure for the sample was 98 mmHg (sd 10 mmHg). During pregnancy, the mothers had been interviewed twice - before 16 weeks and at 30-34 weeks. On both occasions, women completed a dietary assessment, using a food frequency format. Median daily energy intake during pregnancy was about 9 MJ, with median contribution of protein 16-17%, and median calcium intake from food sources of almost 1,000 mg per day. Around 30% of women took supplements containing calcium (median calcium content 75 mg). **Results** In multiple regression analysis, dairy protein content of the mother's diet in late pregnancy was inversely related to the child's systolic blood pressure at 3½ years. Each iso-energetic 1% increase in dairy protein intake was associated with a reduction in child's blood pressure of 0.4 mmHg [95% CI 0.0 - 0.8]. In addition, each 100 mg increment in the mother's daily calcium intake from food sources in late pregnancy was associated with a decrease in child's blood pressure of 0.2 mmHg [95% CI -0.1, 0.4]. These associations were strengthened when analysis was restricted to dietary data deemed most reliable, according to pre-specified criteria. Associations persisted after hypertensive complications of pregnancy, smoking during pregnancy, mother's resting blood pressure, and the child's current weight and calcium intake were taken into account. Contributions of dairy protein and calcium from food sources could not be separated, statistically. Effects were not mediated by birth weight. Antenatal calcium from supplements was not independently associated with child's blood pressure. There were no associations for total protein or carbohydrate content. **Conclusion** These observational data support the proposition that maternal diet during pregnancy has a long-term influence on metabolism of the child. In particular, dairy protein and calcium from food sources appear to be beneficial for blood pressure.

O-033

Low Maternal Vitamin B12 and High Folate Predict Offspring Adiposity & Insulin Resistance at 6y: Pune Maternal Nutrition Study (PMNS) Swapna S Deshpande[1], Chittaranjan S Yajnik[1], Sadanand S Naik[1], Dattatray S Bhat[1], David J Fisher[2], Helga Refsum[3], CHD Fall[2]. 1.Diabetes Unit, KEM Hospital and Research Centre, Pune, India 2. MRC Environmental Epidemiology Unit, Southampton, UK 3. Pharmacology, University of Oxford, UK

Background: Vitamin B12 and folate influence fetal growth and may contribute to intra-uterine programming of type 2 diabetes and cardiovascular disease. Vitamin B12 deficiency is common in Indians. In a preliminary study we have reported an association between high maternal homocysteine concentrations and low offspring birthweight. **Objective:** To investigate the relationship of maternal vitamin B12 and folate status in pregnancy to offspring size at birth and adiposity and insulin resistance 6y of age. **Methods**: We measured maternal plasma vitamin B12, homocysteine (tHcy), methyl malonic acid (MMA) and red cell folate concentrations in 700 pregnant rural women at 18 and 28 wk gestation in the PMNS. We measured offspring size at birth and their body composition and insulin resistance at 6 years of age. **Results**: Median plasma vitamin B12 concentration in these mothers was low: 18 wks gestation 135 pM/L and 28 wks 122 pM/L. Seventy one percent had low vitamin B12 status (<150 pM/L), 90% had high MMA concentration (>0.26 µM) but only one woman had low folate status. Median plasma tHcy concentration was 8.6 µM (IQR, 6.7,10.8). Higher intake of milk and non-vegetarian foods predicted higher vitamin B12 status. Maternal vitamin B12 status was unrelated to neonatal size. Higher maternal folate predicted large neonatal size (adjusted for maternal age, parity, gestation at birth and offspring gender). Higher maternal plasma tHcy concentration was associated with smaller newborn size and an increased risk of a small for gestation (SGA) baby (OR= 2.8, 95% CI 1.18-6.66, p=0.02). Higher maternal vitamin B12 concentration at 28 weeks gestation predicted lower BMI (lowest quartile (q1) mean 13.5 vs highest quartile (q4) mean 13.2 kg/m^2, p=0.008) and lower truncal fat [(q1) 1.0 vs (q4) 0.9 kg, p=0.046) in the child at 6y. Higher maternal red cell folate concentration at 28 weeks predicted higher adiposity in the child [total fat mass ((q1) 3.0 vs (q4) 3.4 kg, p=0.002, as well as trunk and leg fat mass, $p<0.01$ for both adjusted for current size, gender, socio-economic status and maternal size. In a multiple regression analysis, lower maternal vitamin B12 (standardized ß= -0.12), higher folate (standardized ß= 0.17 and higher vitamin C (standardized ß=0.15) concentrations were independent predictors of higher insulin resistance in the child ($p<0.01$ for all). **Conclusion**: This is the first demonstration of an association between maternal nutrient status in pregnancy and offspring adiposity and insulin resistance. Our findings have important implications for nutritional advice to Indian mothers and strategies to prevent obesity and type 2 diabetes.

Parallel Session 3B: Developmental Plasticity

O-034

Attenuation of Glucocorticoid Programmed Hypertension by Postnatal Dietary Omega-3 Fatty Acids: Involvement of the Renin-angiotensin System Caitlin S. Wyrwoll, Peter J. Mark, Brendan J. Waddell; *School of Anatomy and Human Biology, The University of Western Australia, Nedlands, Perth, Western Australia 6907, Australia*

Background. Fetal programming is now recognised as a key determinant of the adult phenotype, with major implications for adult-onset diseases including hypertension. A key mediator of fetal programming is fetal glucocorticoid exposure and recent studies show that postnatal dietary manipulations can exacerbate programming effects. We recently established that postnatal ingestion of omega-3 fatty acids can attenuate glucocorticoid-induced programming of hypertension in six month old rats. This study aimed to determine whether programmed hypertension and its prevention by postnatal dietary omega-3 fatty acids are mediated via the renin-angiotensin system (RAS). **Methods**. Dexamethasone (0.75 µg/ml in drinking water) was administered to pregnant rats from day 13 to term. The offspring of treated and control mothers were cross-fostered to mothers on either a standard or high omega-3 diet, and remained on these diets post-weaning. Systolic blood pressure was measured by tail-cuff plethysmography. In six month old rats, renal angiotensin converting enzyme (ACE), renin, and angiotensin type 1 receptors (AT-1a and b), and hepatic angiotensinogen mRNA expression were measured by real time quantitative RT-PCR. **Results**. Maternal dexamethasone treatment reduced birthweight by 27% and delayed the onset of puberty in offspring. Hypertension was evident in offspring by 6 months of age in dexamethasone-exposed animals consuming a standard diet, but these effects were completely blocked by a high omega-3 diet, with systolic blood pressure 13.2 and 19.3 mmHg lower in male and female offspring respectively. Dexamethasone exposure *in utero* elevated ACE expression by more than two-fold in kidneys of males consuming a standard diet but this effect was prevented by a postnatal diet high in omega-3 fatty acids. A similar effect of dexamethasone was observed in the kidneys of female offspring, except that no dietary effect was evident. *In utero* dexamethasone exposure also elevated renal renin expression in both males and females, but this was not attenuated by a postnatal diet high in omega-3 fatty acids. Renal expression of AT-1a and AT-1b mRNA expression was unaffected by either *in utero* treatment or postnatal diet. Hepatic angiotensinogen mRNA expression was also elevated in female, but not male, offspring exposed to dexamethasone *in utero* but this was not modified by postnatal diet.**Conclusions**. Our data show that hypertension programmed by fetal glucocorticoid exposure is likely to be mediated, in part, by increased renal expression of ACE, and this effect was prevented by a postnatal diet high in omega-3 fatty acids. Dexamethasone programmed rats also exhibited altered gene expression in other components of the RAS but none of these were modified by postnatal diet. These results confirm the involvement of the RAS in programmed hypertension and provide some insight as to how this is attenuated by omega-3 fatty acids.

O-035

Prenatal Hypertension Induced by Plasma Infusion Programs Cardiac Growth and Maturation in Fetal Sheep Sonnet S. Jonker, J. Job Faber, Debra F. Anderson, Kent L. Thornburg, R. Kevin Rodgers, Emmeline F. Hou and George D. Giraud; *Departments of Physiology and Pharmacology, Medicine (Cardiology), and the Heart Research Center, Oregon Health & Science University, and the Portland VA Medical Center, Portland, OR, 97239 USA*

The fetus grows its myocardium by proliferation, setting the number of myocytes it will have for life; thus, if the fetal heart meets a hemodynamic challenge by altering its developmental program, it may have life-long consequences. Systolic stress in the late-term fetal sheep tends to produce a more mature heart containing more and larger myocytes. Chronic infusion of plasma into fetal sheep dramatically increases arterial and venous pressures while decreasing circulating angiotensin II, a known proliferative stimulant of fetal sheep cardiomyocytes. We hypothesized that chronic cardiac load produced by plasma infusion would increase myocyte maturation and myocyte hypertrophy, but not proliferation. **Methods**: Lambs were instrumented and infused with plasma from 130±2 (mean±SD) days of gestation (term 145 days) for either 4 days (n=7) or 8 days (n=8). Controls were age-matched but not plasma-infused (n=8 each group). Myocyte lengths and widths, binucleation rates (an index of maturation), and rates of Ki-67 positivity (an index of cell cycle activity) were measured. **Results**: During plasma infusion, arterial pressure increased in the 4-day group (from 43±3mmHg to 63±4mmHg) and the 8-day group (from 43±3mmHg to 63±2mmHg). Venous pressure increased in the 4-day group (from 3.3±0.6mmHg to 5.1±1.3mmHg) and the 8-day group (3.0±0.9mmHg to 5.8±2.9mmHg). Plasma renin activity decreased in the 4-day group (from 7.8±6.5ng/ml/hr to 1.1±6.5ng/ml/hr) and the 8-day group (from 7.2±3.6ng/ml/hr to 1.5±2.2ng/ml/hr). The table shows the changes in cardiac myocyte growth and maturation (*p<0.05).

4-day Control	4-day Experimental		8-day Control	8-day Experimental	
Binucleate length (um)					
	Left ventricle	81.2 ± 4.0	89.9 ± 6.8*	83.3 ± 4.8	91.2 ± 6.8*
	Right ventricle	89.9 ± 5.7	100.0 ± 6.7*	90.3 ± 3.7	100.7 ± 6.8*
Binucleate width (um)					
	Left ventricle	12.2 ± 0.8	12.7 ± 0.7	12.6 ± 1.2	14.0 ± 1.1*
	Right ventricle	15.2 ± 1.2	15.6 ± 1.8	15.7 ± 1.9	17.8 ± 1.4*
Binucleation (%)					
	Left ventricle	48.4 ± 10.2	43.4 ± 13.1	40.2 ± 11.0	62.6 ± 18.2*
	Right ventricle	45.7 ± 10.6	40.8 ± 13.5	46.3 ± 12.2	67.1 ± 12.8*
Ki67(+) mononucleates (%)					
	Left ventricle	4.2 ± 3.2	9.6 ± 5.2*	4.0 ± 2.3	12.5 ± 7.1*
	Right ventricle	5.7 ± 4.0	13.3 ± 8.1*	5.8 ± 3.2	13.5 ± 8.5*

Conclusions: Cardiac growth and maturation is accelerated in plasma-loaded fetal sheep: 1) Cell cycle activity is an early and sustained response of the late-term fetal sheep heart to chronic load, despite low levels of angiotensin II. 2) Myocyte maturation is not increased at 4 days of load, but at 8 days the percent binucleation is increased. Thus, at 8 days the fraction of cardiac myocytes with proliferative potential is reduced, but the actual number of myocytes in the cell cycle is about doubled. 3) Cardiac myocytes undergo hypertrophy, initially increasing in length followed by an increase in width. We speculate that prenatal cardiac loading may alter the final number of cardiomyocytes in the heart, and their associations with the coronary tree and other myocardial elements. These changes are likely to persist in the mature heart and may affect adult myocardial health.

O-036

Transforming Growth Factor beta Receptor (TGFßR)-1 Inhibits Nephrogenesis in Hypertensive Offspring of Food Restricted Rat Dams John S. Torday[1,2], Mina Desai[2], Virender K. Rehan[1], Michael G. Ross[2]. [1]Department of Pediatrics, [2]Department of Obstetrics and Gynecology, David Geffen School of Medicine at UCLA and LABioMed at Harbor-UCLA Medical Center, Torrance, CA 90502, USA

Background: Maternal food restriction (FR) results in reduced kidney nephron number and in associated with systemic hypertension in the offspring, though the mechanisms underlying this phenotype is unknown. TGFß plays diverse and critical roles in kidney development and is essential for normal development of the kidneys. Decreased TGFß signaling is necessary for normal kidney branching morphogenesis. We therefore hypothesized that maternal nutrient restriction inhibits the physiologic down-regulation of kidney TGFß signaling, causing suppression of fetal/newborn nephrogenesis and impaired renal development. The resulting permanent alterations in kidney structure and function (e.g., reduced number of nephrons) may potentiate a hypertensive phenotype. We determined the mRNA expression of TGFßRs 1, 2 and 3 in fetuses (e16, e0=day of mating) and newborns (day 1) from control and food-restricted rat dams. **Methods:** Pregnant Sprague Dawley rats and offspring were studied. Control dams (n=5) received ad libitum food, whereas study dams (n=5) were either 50% food-restricted (FR) from pregnancy days 10 to 16 or from days 10 to term. Kidneys were excised from fetuses at e16 and from newborns at 1 day. Transforming Growth Factor beta Receptors (TGFßR) 1, 2 and 3 mRNA expression was determined by Real Time PCR (Perkin Elmer ABI PRISM 7700 Sequence Detection System, TaqMan™). The PCR primers for TGFßR 1, 2 and 3 were designed using the primer design program Primer Express (ABI). Internal genes for all the genes probed were also designed using Primer Express. The probes consisted of two tandem oligomers, one of which had attached to it the dye FAM, or VIC (for GAPDH or 18S). The second oligomer had the quencher TAMRA attached (for all probes). A preliminary TaqMan™ reaction was performed to optimize primer/probe concentrations. Real-Time PCR was carried out using primers and probes for both the gene of interest and the internal standard (18S mRNA) in the same reaction. Data were analyzed by Student's t-test. **Results:** At e16, the kidneys of FR fetuses had significantly higher expression of TGFßR 1 (FR 70%> control, $p<0.01$) as compared to Control fetuses. However, there were no significant differences seen in either TGFßR 2 or TGFßR 3 expression between the two groups ($p>.05$), indicating a very specific and selective change in TGFßR. Notably, in 1 day old newborns, no significant differences in TGFßR's between FR and control were evident. **Conclusion:** Maternal FR upregulates TGFßR 1 suggesting an upregulation of fetal TGFß signaling, the 'stop' signal for kidney tubule formation. The resulting inhibition of fetal nephrogenesis results in reduced nephron numbers. This may be the underlying mechanism involved in hypertensive phenotype seen in offspring exposed to maternal nutrient deprivation in utero.

O-037

Maternal Diet 'Programs' Late Onset Mitochondrial Dysfunction in Renal Tissue of Male Offspring Piran Shelley[1], Jane Tarry-Adkins[2], Malgorzata S Martin-Gronert[2], Simon J Heales[1], John B Clark[1], Lucilla Poston[3], Susanne E Ozanne[2], Josie ML McConnell[3]. [1]Division of Neurochemistry, Institute of Neurology UCL, London, UK. [2]Department of Clinical Biochemistry, Addenbrooks Hospital, Cambridge University, Cambridge, UK. [3]Division of Reproductive Health, Endocrinology and Development, St Thomas' Hospital, KCL, London UK.

Reduced intrauterine protein availability followed by adequate nutrition during weaning and adulthood has been shown to influence the adult health of the offspring[1]. We have developed a model of protein deprivation and catch up growth, which demonstrates reduced longevity[2] and increased rates of telomere attrition[1]. We hypothesise that dysfunctional mitochondria could be a contributory factor in these observations[3]. The activities of key mitochondrial enzymes together with ubiquinone (CoQ9 & CoQ10) concentrations were measured in kidney at various ages after birth. Wistar rats were fed a control diet (C) 20% protein during pregnancy and lactation, after weaning the offspring were fed a chow diet, until tissue collection, or a recuperated diet (R) 8% protein during pregnancy, 20% protein during lactation and a chow diet until tissue collection. Citrate synthase, mitochondrial enzyme complex I to IV activities and ubiquinone were measured in the cortex and medulla regions of the kidney at 3 months and 12 months, whole kidney was analysed for the 22 day samples. Results were normalised using protein content of the sample. In the cortex a significant down regulation of mitochondrial respiratory chain enzyme activity, citrate synthase enzyme activity and GSH was observed using a paired t-test in standardised results; citrate synthase; C (136±3.58) vs. R (119±3.37) $p<0.01$, complex II+III; C (41±3.06), R (24+1.29) $p<0.001$, complex II; C (93±3.89) vs. R (83±3.17) $p<0.05$, complex IV; C (3.18+0.27) vs. R (2.41±0.11) $p<0.01$and a significant reduction in CoQ9 C (908.0± 39.3) vs. R (789.4±26.9) $p<0.01$. Both these alterations precede significant differences in telomere length[2]. Results at earlier stages (22 days/3months) showed negligible effects of maternal diet. We conclude that intrauterine protein restriction and catch up

grow causes mitochondrial abnormalities in adult offspring. This could contribute to deficiencies in energy production or increases in oxidative stress leading to telomere attrition and premature death.

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Parallel Session 3C: Diabetes and Metabolic Syndrome

O-038

The Effect of Maternal Body Condition Score during Pregnancy on Glucose Tolerance and Body Composition of Young Male Adult Sheep Offspring

R.L. Cripps[1], L.R. Green[2], J. Thompson[3], M.S. Martin[1], M. Monk[1], M. Sheldon[3], M.A. Hanson[2], C.N. Hales[1] & S.E. Ozanne[1]. *[1]Department of Clinical Biochemistry, University of Cambridge, Box 232, Level 4, Addendrookes Hospital, Hills Road, Cambridge, CB2 2QR, UK, [2]Centre for Developmental Origins of Health and Disease, University of Southampton, Level F (887), Princess Anne Hospital, Coxford Road, Southampton, SO16 5YA, UK, [3]The Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire, AL9 7TA, UK*

Background: Both epidemiological and animal model studies have revealed a link between poor maternal nutrition during pregnancy and increased subsequent risk of type 2 diabetes and the metabolic syndrome in the offspring. Maternal body mass index has previously been implicated in altered offspring cardiovascular control in both fetal and adult life. The effect of maternal body condition on the young adult sheep offspring metabolism has not been studied to date. **Methods**: Two groups of Welsh Mountain ewes were established and maintained, by manipulation of nutrient intake, at a body condition score (BCS, 1-5) of 2 (lower body condition (LBC), n=17) and >3 (higher body condition (HBC), n=19) prior to and during pregnancy. Male offspring biometry, BCS, back fat and muscle depth were measured at birth, 4, 8 weeks, and after weaning at 12, 16, 26, 48 and 73 weeks of age. At 73 weeks of age an intravenous glucose tolerance test was performed and one week later a muscle biopsy was taken from the vastus lateralis muscle. Plasma glucose was measured using an autoanalyser as part of routine assays, plasma insulin was measured using a sheep insulin solid-phase, two site enzyme immunoassay kit and protein expression by Western blotting. Data were analysed using Students's t-test except for insulin and BCS which were analysed using Mann Whitney U test. Data are shown as mean ± SEM except insulin and BCS, which are shown as median (IQR). **Results**: Offspring from LBC and HBC ewes were indistinguishable in terms of physical characteristics from birth to 73 weeks of age aside from reduced muscle depth at 8 weeks of age (23.0 ± 0.61 mm vs. 25.28 ± 1.56 mm, $p<0.01$) and reduced BCS at 26 weeks of age (2.63 (2.50-2.75) vs. 2.75 (2.50-3.00) $p<0.05$) in LBC compared to HBC offspring. At 73 weeks, LBC offspring had increased fasting plasma glucose concentrations (3.8 ± 0.07 mMol vs. 3.6 ± 0.05 mMol, $p<0.05$) and glucose area under the curve during glucose tolerance test (2274 ± 22.6 vs. 2161 ± 33 min*mMol, $p<0.01$). The initial insulin response to the glucose challenge was reduced in the LBC offspring with lower plasma concentrations of insulin at 5 mins after the glucose bolus (208 (128-231) pMol vs. 272 (189-336) pMol $p<0.05$). There were no differences in expression of Insulin receptor, PKCζ, IGF1 receptor or p85 subunit of P13 kinase in the vastus lateralis muscle between the two groups. **Conclusions**: This study shows that reduced maternal BCS during pregnancy, within the normal range, alters glucose metabolism of the young adult offspring independent of birth and current weight. The reduced first phase insulin secretion is thought to be an early indicator of the development of type 2 diabetes. *Supported by NIH*

O-039

***In Vivo* Mitochondrial Function in Young Men Born with Low Birth Weight, as Assessed by ^{31}P-NMR Spectroscopy during Rest and after Energy Depleting Exercise**

Charlotte Brøns[1,2], Julie Solbjerg Appel[1,2], Christine Bjørn Jensen[2], Heidi Storgaard[2], Arne Astrup[1], Bjørn Quistorff[3], Allan Vaag[2]. [1]Department of Human Nutrition, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark; [2]Steno Diabetes Center, Gentofte, Denmark; [3]Department of Medical Biochemistry and Genetics, Panum Institute, Copenhagen, Denmark.

Background. Low birth weight (LBW) is associated with increased risk of insulin resistance and development of type 2 diabetes (T2D) later in life. The underlying molecular mechanism(s) remain largely undetermined. Recent magnetic resonance spectroscopy studies in humans suggest that even subtle defects in mitochondrial function may play a role in the pathogenesis of (muscle) insulin resistance in aging and T2D. Mitochondrial dysfunction in the liver and pancreatic beta cells has also recently been linked with impaired insulin secretion in growth retarded rats, indicating a general intrauterine programming mechanism. The aim of this study was to determine whether LBW in humans is associated with impaired mitochondrial function in skeletal muscle tissue during rest and after exercise. **Methods**. We evaluated *in vivo* mitochondrial function in the forearm flexor muscle by ^{31}phosphorus nuclear magnetic resonance spectroscopy (^{31}P NMR) in 18 24-year old healthy lean men with LBW (BW = 10th percentile) and 15 matched normal birth weight (NBW) (50th = BW = 90th percentile) controls. There was no significant difference between the groups with regards to BMI, W/H, fat mass and lean mass; however fasting b-glucose was significantly higher in LBW subjects 4.59±0.43 vs. 4.99±0.48 mmol/L (P=0.003). In addition, identical measurements were obtained from the gastrocnemius/soleus muscle in a subset of the original population (LBW N=12, NBW N=8). A standard exercise protocol was performed with 4 successive maximal voluntary contractions each lasting 30 sec interspersed by a 60 sec recovery period. The ^{31}P NMR technique provides non-invasive real-time estimates of resting state parameters of energy metabolism as well as mitochondrial aerobic capacity.
Results. The LBW subjects had a significantly lower resting forearm muscle PCr/Pi ratio (8.04±1.36 vs. 9.70±1.66, P=0.004), and a non-significantly lower resting forearm PCr level (21.57±1.90 vs. 22.74±2.24 mM, P=0.10) and PCr/ATP ratio (3.93±0.34 vs. 4.14±0.40, P=0.10), when compared to NBW controls. In addition, the LBW subjects had a lower initial rate of PCr synthesis (0.09±0.05 vs. 0.14±0.06 mM/s, P=0.02) and a longer recovery of PCr ($t_{½}$) (75.6±26.1 vs. 52.6±15.4 sec, P=0.005) after exercise, while there was no significant difference in the aerobic capacity. Finally, LBW subjects had lower pH at the end of the exercise (6.34±0.28 vs. 6.55±0.30, P=0.04). None of the above differed significantly in calf muscle as a function of birth weight. **Conclusion**. The data documents a depletion and altered metabolism of muscle high energy phosphorous compounds in 24-year old healthy men with low birth weight. The results thereby support the idea of mitochondrial function being programmed during fetal life. Importantly, as these changes occur in young, lean non-diabetic subjects, we speculate that mitochondrial dysfunction may play a primary role in the pathogenesis of muscle insulin resistance and T2D associated with low birth weight.

O-040

More Centrally Obese Adolescent Indian Children have Evidence of Insulin Resistance in Early Childhood – Pune Urban Cohort Study

Ashish R Bavdekar, Madhumati S Otiv, Sheila A Bhave, Anjali S Mote, Vaishali A Madkaikar, Anand N Pandit, Chittaranjan S Yajnik, Charu V Joglekar, Caroline HD Fall, Department of Pediatrics and the Diabetes Unit, King Edward Memorial Hospital, Pune, India, Medical Research Council, Environmental Epidemiology Unit, University of Southampton, UK.

Background: In 1995 we reported an inverse relationship of birth weight with post glucose load 30 min glucose and insulin at 4 years of age in an urban Indian cohort (n=190). A follow up study of this cohort in 1999 at 8 years of age with additional 287 children demonstrated that children with the most adverse cardiovascular risk profiles were light at birth and heavy at 8 years. Of the components of body weight, higher fat mass was associated with an increase in all the risk variables. This cohort has been followed annually for growth, anthropometry, and has now reached ages of 16-17 years. This purpose of this study is to determine if more centrally obese adolescent children have any evidence of increased cardiovascular risk factors in early childhood. **Methods:** This study is restricted to children with known birthweight who were studied at 4 years, 8 years and now at 16-17 years. The parameters studied in these children at 4 and 8 years of age were anthropometry (weight, height, BMI, head circumference, waist circumference, skin fold thickness and calculated fat mass), biochemistry (fasting and 120 mins blood glucose and insulin after a glucose load, calculated insulin resistance (HOMA-R), leptin (at 8 years), serum cholesterol, triglycerides, HDL cholesterol), socioeconomic status and parental anthropometry. At 16-17 years, detailed anthropometry was taken including measures of adiposity like BMI (generalized adiposity) and waist circumference (central obesity). Relationship of birthweight, 4 and 8 year variables with these measures of adiposity were analyzed. **Results:** 137 children fulfilled the inclusion criteria. There were 74 boys and 63 girls. Mean age of the children at last visit was 16.47 (SD 0.48) yrs. Children who were in the highest tertile of BMI at 16-17 years had significantly higher weight ($p=0.04$) and waist circumference ($p=0.003$) at 4 years than the rest. No association was seen with birthweight, lipids, fasting and 120 min glucose insulin values and HOMA-R. At 8 years, this group had higher BMI, fat mass, HOMA, leptin, fasting insulin at 8 years. Both their parents also had higher BMI and waist circumferences (all $p < 0001$). Children in the highest tertile of waist circumference at 16-17 years had significantly higher BMI ($p=0.05$), weight ($p=0.01$), waist circumference (0.0001), HOMA-R ($p=0.03$) and 120min glucose ($p=0.05$) at 4 years. They also had higher BMI, fat mass, HOMA, leptin, triglycerides, and fasting insulin at 8 years. Both their parents also had higher BMI and waist circumferences (all $p < 0.0001$). **Conclusions:** In the more centrally obese adolescent children, evidence of insulin resistance could be detected as early as 4 years of age. Adolescent children with higher generalized obesity did not show this association. However at 8 years both these groups had adverse cardiovascular risk profiles.

O-041

The Associations of Birth Weight and Childhood Body Mass Index with Diabetes in a Large Cohort of Individuals Born in the 1950s

Debbie A Lawlor & David A Leon. *Department of Social Medicine, University of Bristol, UK; Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, UK*

Background Studies of the association between birth weight and adult diabetes have tended to be small and largely conducted in populations that were born in the 1930s or earlier in developed countries. There is considerable concern that the current childhood obesity epidemic will result in increases in type 2 diabetes. However, few studies have examined the association of childhood body mass index (BMI) with future diabetes risk. **Methods** We examined the associations of birth weight and childhood BMI with

adult diabetes risk in a large birth cohort of participants born in Aberdeen, Scotland between 1950 and 1951. Perinatal data were obtained from the Aberdeen Maternal and Neonatal Database having been collected during the perinatal period using research criteria. Childhood anthropometric details were obtained from school entry examinations. We used two measurements of adult diabetes: responses to a questionnaire mailed to all surviving participants in 2000 (N = 7,148 (63%) responded) that asked about doctor diagnosis of diabetes and its treatment and hospital admissions data from the Scottish Morbidity Register from which we had information on all hospital admissions for 10,803 participants who were singleton births and alive and still living in Scotland on 1st January 1981 (the date from which the hospital admissions data is available). **Results** For individuals with both questionnaire and hospital admissions data there was consistency in diabetes diagnosis and the associations between early life exposures and later disease were essentially the same which ever of these two outcomes were used. The odds ratio of diabetes for a 1SD (sex and gestational age standardised z-score) increase in birth weight was 0.77 (95%CI: 0.65, 0.92) and that for a 1SD (sex and age standardised z-score) increase in BMI at school entry (mean age 5 years) was 1.19 (1.03, 1.37). The association with both of these exposures was linear across their distribution. Of note, there was no evidence of a reverse 'j' shaped association for birth weight and no evidence that the association with childhood BMI was driven by increased risk only in the obese category. Adjustment for a range of indicators of childhood socioeconomic position (father's occupational social class, maternal age, marital status and height at birth) and maternal complications during pregnancy or labour did not substantively alter either of these associations. Adjustment for adult BMI had very little effect on the association with birth weight but the association with childhood BMI attenuated to the null with adjustment for adult BMI. There was no evidence of any interactions between birth weight and either childhood or adult BMI (p-values all >= 0.2), but birth weight and either childhood or adult BMI combined independently to increase diabetes risk. Thus, individuals in the lowest birth weight quarter and highest adult BMI quarter had the greatest risk of diabetes (7.0% (95%CI: 4.6, 10.2%)) and those in the highest birth weight quarter and lowest adult BMI quarter had the lowest risk (0.2% (95%CI: 0.4, 3.1%)). **Conclusions** Birth weight is inversely associated with diabetes, in a population born at a time when environmental circumstances, as indexed by low infant mortality rates, were relatively advantageous for infants. This association is not confounded by socioeconomic position or maternal complications of pregnancy. Adult BMI appears to be a more important determinant of diabetes risk than childhood BMI.

Parallel Session 3D: Programming of Nutrition and Physical Activity

O-042

Prenatal Dexamethasone Induces Metabolic Changes in Chlorocebus aethiops (Vervet) Offspring Annick de Vries[1], Megan Holmes[1], Jürgen V. Seier[2], Joritha van Heerden[2], Johan Louw[2], Sonia Wolfe-Coote[2], Naomi S. Levitt[3] and Jonathan R. Seckl[1]; [1] Endocrinology Unit, Centre for Cardiovascular Science, The Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK, [2] Primate Unit, Diabetes Research Group/MRC, Tygerberg, Cape Town, South Africa, [3] Medical Sciences Discipline, University of the North, Polokwane, South Africa

Antenatal steroid treatment is widely used for the prevention of neonatal respiratory distress syndrome in preterm infants. However, like stress and the associated increases in glucocorticoids, artificial steroids given antenatally affect the offspring detrimentally later in life. This study focuses on the effects of prenatal stress hormone application in the non-human primate Chlorocebus aethiops (vervet). Thereby it provides a link between rodent studies on glucocorticoid programming and the clinic. In our model, pregnant vervet mothers were administered dexamethasone from mid-term onwards, daily, at 50, 120 or 200 µg/kg, producing offspring denoted Dex50, Dex120 or Dex200, respectively. At 8 months of age, Dex vervet offspring were hyperinsulinaemic and displayed slower glucose clearance of a glucose load. Dex120 showed an increase in basal insulin, whereas all prenatally dexamethasone exposed vervets showed an increased insulin response 10 min after glucose administration compared to controls. There was no change in basal glucose, nor peak glucose in response to a glucose load in any Dex offspring. However, slower glucose clearance was found in Dex120 and 200 as glucose levels were higher than controls between peak up until 60 mins after glucose administration. These changes in insulin and glucose were reflected in changes in the pancreas: a reduction in the number of ß cells as well as a smaller size of ß cells in Dex120 and 200 compared to controls. Furthermore, consistent with glucocorticoid programming in humans and rodent models, we observed blood pressure increases due to prenatal dexamethasone occurring when the offspring were 12-14 months old (systolic blood pressure was increased from 83.1 ± 2.2 in controls to 92.5 ± 3.7 mmHg in the Dex120 vervets). Mild stress, induced by blood sampling, produced an exaggerated rise in plasma cortisol levels of Dex200 compared to controls. Cortisol regulates its own secretion by exerting a negative feedback signal on the hypothalamus and pituitary. As we had observed elevated cortisol levels, we postulated that there may be a dysfunctional feedback in the Dex animals. To test this we carried out a Dexamethasone suppression test, but observed no differences between controls and Dex vervets. Moreover, it seemed there was actually an increased feedback or an increased 'forward drive' in Dex200, as Dex200 showed significantly lower levels of serum cortisol compared to controls in the dexamethasone suppression test. We did not find any changes in cortisol and its metabolites in 24 hr urine samples indicating there was no change in basal glucocorticoid metabolism. Overall, we conclude that prenatal dexamethasone in the non-human primate Chlorocebus aethiops gives rise to metabolic and cardiovascular changes similar to changes found in the rodents after prenatal dexamethasone. This study provides a clear link between fundamental research in rodents and the reported effects in the clinic on high dexamethasone administration in utero. The detrimental effects described here are likely to affect the offspring in a way that could give rise to diabetes and long term hypertension in the adult. This work is supported by the Wellcome Trust.

O-043

High Maternal Pregnancy Weight Gain is Associated with an Increased Risk of Obesity in Childhood and Adulthood Independent of Maternal BMI

Lene Schack-Nielsen[1], Erik L. Mortensen[2], Kim F. Michaelsen[1], Thorkild I.A. Sørensen[3]; [1]Department of Human Nutrition and Centre for Advanced Food Studies, The Royal Veterinary and Agricultural University DK-1958 Frederiksberg C, [2]Department of Health Psychology, Institute of Public Health, University of Copenhagen, DK-2200 Copenhagen N, [3]Danish Epidemiology Science Center, Institute of Preventive Medicine, Copenhagen University Hospital, DK-1357 Copenhagen K, Denmark

Background: Maternal pregnancy weight gain (PWG) is positively associated with birth weight (BW) that is positively associated with BMI in childhood and adulthood, but whether PWG has a long term effect on offspring BMI independent of BW and maternal BMI is not known. **Methods**: The Copenhagen Perinatal Cohort consists of 9125 individuals born at the Copenhagen University Hospital in 1959-1961. Information on BW, gestational age (GA), socioeconomic status (SES) at birth, smoking during pregnancy, duration of breast feeding, maternal age, weight, height and PWG (in 5 categories assigned the interval middle value) were recorded prospectively at birth and at a 1 y examination. Weight and height were available from follow-up examinations at 1, 3, and 6 y, from school health records at 8, 11, and 14 y and from a mailed questionnaire at the age of 42-44 y. BMI measurements and BW were transformed into z-scores also adjusting BW for GA (The British Growth Reference 1990). Regression analysis with BMI in childhood or adulthood as dependent variable was performed. For 3426 participants information was available to be included in at least one analysis. **Results**: The prevalence of overweight (BMI>25.00 kg/m^2) and obesity (BMI>30.00 kg/m^2) were 9 and 1% among the mothers and 43 and 11% among the offspring as adults. PWG was 5.5, 7.0, 9.5, 11.5, 14.0 and 16.0 kg for 7, 16, 18, 18, 21, and 19 % of the mothers, respectively. Maternal BMI was higher in the lowest PWG group compared to the others (BMI±SD, 22.7±3.8 vs. 21.4±2.6, P<0.001). In regression analyses adjusted for BW z-score, maternal age, SES, smoking during pregnancy, sex both maternal BMI and PWG were positively associated with BMI z-scores at all ages as shown in the table.

	BMI z-score					
	1 y (n=2034) ß (95% CI)	3 y (n=1408) ß (95% CI)	6 y (n=1070) ß (95% CI)	8 y (n=1037) ß (95% CI)	14 y (n=940) ß (95% CI)	42-44 y (n=1660) ß (95% CI)
Maternal BMI (kg/m^2)	0.05 (0.03-0.07)	0.06 (0.03-0.08)	0.07 (0.05-0.10)	0.08 (0.06-0.10)	0.10 (0.08-0.13)	0.12 (0.10-0.14)
PWG (kg)	0.03 (0.02-0.05)	0.02 (0.00-0.04)	0.02 (0.00-0.04)	0.03 (0.02-0.05)	0.03 (0.01-0.05)	0.03 (0.01-0.04)

There were no indication of interaction at any ages between maternal BMI and PWG as tested by comparing underweight (BMI=20.00 kg/m^2) and normal weight (BMI>20.00 kg/m^2) or normal weight and overweight mothers. In logistic regression PWG was associated with an increased risk of adult obesity OR (95% CI) 1.05 (1.01-1.10) per kg. Including duration of breast feeding in these models showed a negative effect on 1 y BMI (ß (95% CI) -0.02 (-0.04-0.00) 1 y BMI z-score per months of breast feeding), but no effect at any later age. This did not change the estimates for the effect of maternal BMI and PWG. **Conclusion**: Our data suggest a long term effect of PWG on offspring BMI that is independent of BW, maternal BMI and duration of breast feeding. This was seen in a cohort with a low maternal prevalence of overweight and obesity, whereas the prevalence among the offspring was much higher. A lower PWG is recommended in overweight or obese mothers, which may have a positive effect on the risk of obesity in the offspring beyond the effect on BW.

O-044

Physical Activity is the Real Fat Controller David S. Gardner, Michael E. Symonds; *Centre for Reproduction and Early Life, School of Human Development, University Hospital, Nottingham, UK.*

Background: Whether adult obesity predominantly reflects gluttony or sloth is much debated. However the facts are clear: 1) the prevalence of obesity is rapidly increasing in westernised populations; 2) energy consumed as food on a daily basis in these populations has fallen in the last 50 years but 3) energy expended in physical activity on a daily basis has fallen further. Sub-threshold physical activity levels fail to break deleterious feast-famine metabolic cycles resulting in excess energy deposition as fat (Chakravarthy & Booth 2004). Indeed even minor differences in non-exercise related physical activity may, over time, have a large impact on body weight regulation, and such differences are proposed to have an early-life origin (Levine *et al* 2005). In the current study ambulatory physical activity and 24h energy intake have been measured in sheep in differing environments and related to their current weight. **Methods:** At day 23 gestation 37 twin-bearing ewes were randomly allocated to receive either a control (C, 7 MJ/day; n=24) or nutrient restricted diet (NR, 50% C intake ~3.5 MJ/day; n=13) from day 30 to 80 gestation. Thereafter all sheep were fed to 100% calculated metabolisable energy requirements to term (12-13 MJ/day near term). Offspring delivered spontaneously and were either ewe reared or bottle-fed (BF, n=8 from

control group; 1-1.5 L/d Volac) until weaning. From weaning to 1yr of age offspring were either group-housed in a barn (restricted activity) with increased energy dense food available to promote fat deposition (Obese controls OC, n=8; Obese nutrient restricted ONR, n=13; Obese bottle-fed OBF, n=8) or pasture grazed (unrestricted activity; Lean controls LC, n=8). At one year of age all offspring were weighed and humanely euthanased (electrocortical stunning with exsanguination). Physical activity was measured over 24h periods using a uniaxial accelerometer (Actiwatch, Cambridge Neurotechnology, Cambridge, UK) attached via a collar around the neck of the sheep. Sampling rate was every 30secs. All animal protocols and procedures were locally approved and performed under the UK Animals (Scientific Procedures) Act, 1986. **Results:** At 6 months of age when physical activity measurements were first undertaken pasture grazed sheep (LC) had significantly greater (3-4 fold) activity than sheep housed within a barn (Table). When these sheep were barn-housed i.e. had similarly restricted activity for a week, their activity remained 2-fold that of all other obese groups. In contrast, when barn housed sheep were pasture grazed for a week their activity did not increase significantly above that observed when they were confined in a barn, with the exception of bottle-fed sheep (Table). Physical activity differences between groups remained when the sheep were individually housed within an experimental Home Office designated holding pen. There was a reciprocal relationship between bodyweight and 1) activity during the light phase (r= ¯0.28, *P*=0.04), and 2) variance in activity (r= ¯0.33, *P*=0.01). In addition the rank order of daylight activity was maintained from 6 to 12 months of age (r=0.52, *P*=0.002).

	Lean	Obese			
Activity (units x 10^3)	LC	OC	ONR	OBF	*P*
... in Field	483 ± 60^a	118 ± 36^b	116 ± 14^b	145 ± 30^b	0.000
... in Barn	198 ± 41^a	118 ± 25^a	93 ± 7^b	97 ± 10^b	0.007
... in Exp Holding Pen	170 ± 20^a	61 ± 7^b	67 ± 9^b	96 ± 16^b	0.000

Key: Values within a row not sharing a superscript are significantly different (1-way ANOVA).

Conclusions: In this study sheep grazed at pasture from weaning to 12 months of age had ~3.5 fold the activity of barn housed sheep and consequently were 60% the weight of barn housed at this time. The greater activity of these sheep remained when they were brought indoors. Indeed the rank order of activity for all sheep was maintained from 6 to 12 months of age indicating that the early environment (i.e. <6 months in this study) influences voluntary low-moderate activity levels and ultimately body weight. *This study was funded by the British Heart Foundation and the University of Nottingham.*

O-045

The Nutritional Environment During Lactation is Critical for Programming Postnatal Growth and Adult Body Composition Following Placental Restriction in the Rat Mary E. Wlodek[1], Amy L. Mibus[1] Rachael O'Dowd[1], Kerryn T. Westcott[1] and Julie A. Owens[2]. [1]*Department of Physiology, University of Melbourne, Parkville, Victoria, 3010;* [2]*Gynaecology and Obstetrics, University of Adelaide, Adelaide, 5005*

Background: Fetal growth restriction followed by accelerated growth in infancy and childhood is implicated as contributing to the increased risk of developing hypertension, diabetes and obesity in the adult. In Western society, impaired uteroplacental blood flow is the major characteristic of human pregnancies complicated by intrauterine growth restriction. We have previously shown that placental restriction in the rat impairs fetal growth and mammary function, pup milk intake and growth after birth. Postnatal nutrition and the lactation environment have recently been implicated as critical to the programming of growth and adult disease. We therefore determined, by cross-fostering, the separate influences of prenatal placental restriction and postnatal lactational restriction on postnatal growth and adult body composition. **Methods:** Bilateral uterine vessel ligation (Restricted, R) or Sham (Control, C) surgery was performed on day 18 of gestation in Wistar Kyoto rats (approved by University of Melbourne Animal Ethics Committee). Cross-fostering one day after birth generated six groups: control pup on a control mother (C-on-C); control-on-restricted (C-on-R); reduced litter control (RED)-on-control (RED-on-C); reduced litter-on-restricted (RED-on-R); restricted-on-control (R-on-C); restricted-on-restricted (R-on-R). Male pup weights and dimensions were measured from 3 days to 22 weeks of age. Milk intake during lactation, using the weigh-suckle-weigh protocol, and food intake from week 5 were measured. Male offspring were killed at 6 months (mo) of age and body composition determined. Data were analysed by ANOVA. **Results:** Restricted male pups were born lighter with smaller litter sizes than Control pups ($p<0.05$). Restricted pups who suckled on lactationally restricted mothers (R-on-R) had a lower milk intake during lactation (-60%) and food intake at week 7 compared to C-on-C and grew more slowly (-~25%, $p<0.05$). This appears to reflect both small size at birth and restricted lactation as reduced litter size controls (normal birth weight) suckled on Restricted mothers (RED-on-R) also had a reduced milk intake and increased food intake at week 7 but similar weights to C-on-C. At 6 mo, the R-on-R pups were smaller (-11%), shorter and leaner (-15% total body fat) than both C-on-C and RED-on-R pups ($p<0.05$). Placentally restricted pups cross-fostered onto a mother with normal lactation (R-on-C) had higher milk intake (+40%) and pup body weight compared to R-on-R ($p<0.05$) during lactation. The improved lactational environment increased body weight (+9%) and crown rump length and the proportion of body fat (in omental, dorsal and epididymal fat) of R-on-C compared to R-on-R, ($p<0.05$) at 6 mo. Pups with normal fetal growth and normal litter size but fostered on placentally restricted mothers (C-on-R) had lower milk intake and food intake at week 7 without profound effects on body weight or composition at 6 mo compared to C-on-C. **Conclusions:** Male pups who are born small and are reared in a reduced lactation environment (R-on-R) continue this low growth trajectory without the development of adult obesity, although they develop hypertension, as we have reported elsewhere. Correcting the poor lactation environment prevents this, but promotes accelerated growth and also increases adiposity. Whether a compromised postnatal nutrition for a pup born of normal weight conversely programs cardiovascular dysfunction without adverse effects on body composition awaits further study. These outcomes clearly identify the lactation environment as a critical programming period for growth and disease development.

Parallel Session 3E: Epidemiologic / Nutritional Transition

O-046

Households with Undernourished Children and Overweight Mothers: Is this a Concern for Haiti? Darline Raphael[1], Helene Delisle[2]. [1]*Save The Children US, # 18, rue Emerikc (Imp. Lajoie) Bourdon Petion-Ville, Haiti,* [2]*Department of Nutrition, Faculty of Medicine, Université de Montréal, PO Box 6128 Downtown Station Montreal (Qc) Canada H3C 3J7*

The nutrition transition underway in developing countries (DCs) is in part responsible for the coexistence of undernutrition and obesity. This phenomenon is even more alarming when it happens within the same household. Specific approaches must be found to effectively and simultaneously fight against two apparently opposite nutritional problems, although they share common determinants. The **objectives** of this study were to assess households' nutritional profile in order to determine the prevalence of "malnourished child-overweight/obese mother" households in a poor urban environment in Haiti, and to compare households in relation to the nutrition transition. The **hypotheses** were that the coexistence of child undernutrition and maternal overweight in the same household was related to (qualitative) food insecurity owing to households' poverty and was more frequent in the households having recently settled in the city. **Methodology:** The cross-sectional study included 203 households randomly chosen in a large Haitian shantytown. To take part in this study, households had to include the mother and at least two biological children less than 10 years of age, with one between 6 and 59 months. Recruitment was done through home visits. **Results:** 14% of households had a malnourished child and an overweight mother. Irrespective of child nutritional status, overweight mother households (31%) had better socio-economic status (SES), food security and food diversity scores than those with both malnourished mothers and children (7%), or with malnourished children only (36%). The length of urban residence was not related to the nutritional profile of the household. In **conclusion**, maternal overweight and child undernutrition are both common (31% and 36% respectively) in this Haitian shantytown, which is conducive to the coexistence of child undernutrition and maternal overweight in the same households. The households with better SES, food security and food diversity scores also had higher rates of maternal overweight/obesity. These data suggest that in poor urban settings such as the site of this study, the effects of rapid nutrition transition are still mainly felt in the relatively better-off households.

O-047

Maternal Overweight Concurrent with Child Stunting in Poor Families from Urban Mexico Lynnette Neufeld, Jef Leroy, J Rivera; *National Institute of Public Health, Cuernavaca, Mexico.*

Background: According to a nationally representative sample, the prevalence of overweight among women of child bearing age in Mexico increased from 24.0% to 35.2% from 1988 to 1999. Over the same period, the prevalence of obesity increased by 15% (9.4% to 24.4%). In 1999, more than 27% of children were stunted. While there is a dramatic reduction in the prevalence of stunting with increased socio-economic status in Mexico, there is very little difference in the prevalence of overweight and obesity over deciles of income. The objective of this analysis was to document the coexistence of overweight and/or obesity among mothers and stunting in their children from a sample of poor families from urban Mexico. **Methods:** Participants in this study are beneficiaries of a large national poverty alleviation program and part of a panel to evaluate the impact of the program on nutrition and health indicators. To be eligible for the program, families undergo an economic evaluation and must be below the 20th percentile of income. The sample was recruited in 2002 from urban areas in 16 states in Mexico. A follow-up visit was conducted in 2004. For this analysis, we selected all mother-child dyads who participated in the 2002 and/or the 2004 evaluation. Weight and height were measured at both occasions by highly trained and standardized field workers using standard methods with equipment calibrated daily and were used to calculate body mass index (BMI). Data are treated as cross-sectional for the current analysis and co-occurrence of maternal overweight/obesity and child stunting reported. Overweight, obesity and stunting were defined using current WHO recommendations. **Results:** We identified 1123 mother-child pairs who had participated in the 2002 evaluation and 1506 in the 2004 evaluation (1046 participated in both). In 2004, mean maternal age was 29.8±6.3 years and child age was 35.9±7.5 months. In mothers, the prevalence of overweight and obesity was significantly ($p<0.001$) higher in 2004 than in 2002 (2002: overweight 38.9% obesity 20.2%; 2004: overweight 39.0% obesity 27.0%). Stunting was recorded for 279 (24.8%) children in 2002. The prevalence of stunting among children of women with normal weight, overweight or obesity did not differ from the overall prevalence (24.6%, 24.3%, and 26.4%, respectively; $p=0.8$). **Conclusions:** Overweight and obesity are serious public health problems in Mexico, even among the

poor. At the same time, the prevalence of stunting in children less than 5 years of age remains high and co-exists in mother-child dyads. It is essential that poverty alleviation programs in Mexico address both of these issues simultaneous and ensure that actions taken to improve one of these outcomes do not have negative implications for the other.

O-048

Size at Birth, Infant and Childhood Growth and Adult Body Size and Composition: A Prospective Study in a Stunted Population Corvalán C,[1]Gregory CO,[1] Ramirez-Zea M,[2] Martorell R,[1,3] and Stein AD.[1,3] *Nutrition and Health Sciences Program, Emory University, Atlanta, GA, [2]Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala, [3]Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, GA.*

Background: Excessive body mass, and particularly abdominal fat, is associated with increased risk of diabetes, cardiovascular disease, and some cancers. There is evidence that prenatal and postnatal growth are associated with adult body size and composition, however the relative importance of growth in different time periods is still unclear. **Objective:** To determine the associations among size at birth, infant and childhood growth, and adult body mass index (BMI), fatness, and fat distribution. **Methods:** We studied 309 women and 291 men measured as children between 1969 and 1977 in 4 villages in Guatemala, and re-measured as adults in 2002-4 (mean age 32.7 years (y)). We determined the association of size at birth, infant growth (size at 3y, adjusting for size at birth), and childhood growth (size at 7y, adjusting for size at 3y) with BMI, percentage of body fat (PBF: calculated using predictive equations from hydrostatic weight measurements in a similar population), and abdominal circumference (AC). BMI, PBF, and AC were modeled as continuous outcomes using generalized estimating equations to account for sibling clustering. All models were adjusted for village of birth, year of birth, current residence, and in women, parity. The AC models were also adjusted for adult height. **Results:** Compared to the U.S. population (NCHS 1977), the prevalence of stunting (HAZ<-2) at birth, 3y, and 7y was 48.2%, 74.8%, 80.3% in women, and 45.0%, 69.1%, and 82.5% in men, respectively. Adult overweight (BMI=25 kg/m^2) and obesity (BMI=30 kg/m^2) were more prevalent in women than in men (62.5% and 39.2 % vs. 22.7% and 11.0 %, respectively). In women, larger size at birth and increasing BMI during infancy were associated with higher adult BMI and PBF. In both women and men, an increase in BMI during childhood was associated with higher adult BMI, PBF, and AC. Postnatal linear growth had little association with adult body composition (table).

Early Life Predictors		Adult size and body composition: BMI (kg/m^2) Women	BMI (kg/m^2) Men	PBF (%) Women	PBF (%) Men	AC (cm) Women	AC (cm) Men
BMI (kg/m2)	Birth n=382	**0.63†**	0.07	**0.9***	-0.24	1.02	-0.23
	Infant (0-3 y) n=218	**0.78***	0.43	**1.22***	0.50	1.18	0.79
	Childhood (3-7y) n=218	**2.49‡**	**1.49†**	**3.43‡**	**2.02***	**4.44†**	**3.29†**
HEIGHT (cm)	Birth n=382	**0.46†**	-0.01	**0.86†**	-0.07	0.80	-0.23
	Infant (0-3 y) n=218	0.19	-0.05	**0.45***	0.02	0.67	0.15
	Childhood (3-7 y) n=218	-0.08	-0.15	-0.02	-0.41	-0.17	-0.43

*p<0.05; † p<0.01; ‡ p<0.0001

Conclusions: In our sample, men are leaner than women and hence our observation of few effects among men may reflect reduced power. Nutrition assistance programs targeted at infants, which are effective in ameliorating stunting, appear not to considerably predispose to development of excess adiposity. Post-infancy nutrition programs should be implemented with caution, in order to avoid potential detrimental effects on adult body composition. Funding: NIH (TW005598).

O-049

The Metabolic Syndrome and Associated Lifestyle Factors Among Young Indian Adults B Antonisamy[1], P Raghupathy[2], CHD Fall[3], FS Geethanjali[4], G Priya[1] and J Richard[1]; Departments of [1]Biostatistics, [2]Child Health, and [4]Clinical Biochemistry, Christian Medical College, Vellore, India; [3]MRC Environmental Epidemiology Unit, Southampton, UK.

Background: India is experiencing an epidemic of type 2 diabetes and cardiovascular disease, with an increasingly younger age of onset. Metabolic syndrome or insulin resistance syndrome is considered a strong risk factor for the development of coronary artery disease. Recent studies showed increased prevalence of the metabolic syndrome with high cardiovascular morbidity and mortality. This syndrome is particularly common among Indians, although, in general, they have a lower body mass index as compared to the Western population. Early recognition of the metabolic syndrome and the appropriate management is essential to prevent advent of cardiovascular complications later. **Objective:** This paper presents the current prevalence of metabolic syndrome and examines its relationship to early and current lifestyle factors in a large birth cohort of young Indian adults. **Methods:** 2218 men and women (mean age 28 years) were studied from a population-based birth cohort of 10,670 individuals born during 1969 to 1973 in Vellore town and nearby rural areas. Family history, socioeconomic status, physical activity, tobacco and alcohol use were recorded. Subjects underwent standard oral glucose tolerance test. The metabolic syndrome is defined as per WHO criteria. Logistic regression was used to examine the relationship between lifestyle factors and metabolic syndrome. Mean Z - score of BMI calculated at birth (ponderal index), infancy, childhood, adolescence and adulthood for all subjects who developed metabolic syndrome were compared with those of the others who did not. **Results:** The subjects' median adult BMI was 20.0 kg/m^2. The overall prevalence of metabolic syndrome was 7.6%. (rural-5.3%, urban-10.3%, p<0.001). Men had higher prevalence of metabolic syndrome than women (9.7% versus 5.2%, p<0.001). The prevalence of obesity (BMI =25 kg/m^2) was 8.6% in rural men, 8.8% in rural women, 16.7% in urban men and 26.1% in urban women. The metabolic syndrome in men was associated with higher body fat percent and increased alcohol consumption after adjusting with other lifestyle factors. In women, it was associated with higher body fat percent and increased subscapular:triceps skinfold ratio. Those who developed metabolic syndrome had a low mean BMI Z-score in childhood and an accelerated increase in mean BMI Z-score between childhood and adulthood compared with rest of the cohort (p<0.001). Birth weight did not show any relationship with the occurrence of metabolic syndrome in both men and women after adjusting for adult lifestyle factors. **Conclusion:** Our data suggests high prevalence of metabolic syndrome in young Indian adults, with urban prevalence almost twice as the rural group. Obesity is more prevalent in urban women, thrice as in rural women. Modification of lifestyle factors with a balanced dietary intake and regular physical activity from childhood is likely to be beneficial in preventing the onset of metabolic syndrome.

Parallel Session 4A: Stress / Infection

O-050

Prenatal Psychosocial Stress Alters Immune Cell Population after a Psychosocial Stress Test in Women [1]Sonja Entringer, [1]Robert Kumsta, [1]Dirk H. Hellhammer, [2]Pathik D. Wadhwa, [1]Stefan Wüst; [1]*Department of Psychobiology, University of Trier, Germany; [2]Department of Psychiatry and Human Behaviour, University of California, Irvine, USA*

Background: Maternal stress during pregnancy can result in permanent behavioral, metabolic and immunological changes in the offspring. It was suggested that these effects are mediated by high levels of maternal hormones, in particular glucocorticoids, secreted in response to stress. The present study investigated whether exposure to severe psychosocial stress during pregnancy can have long lasting effects on the immune function of the adult offspring. **Methods**: Thirty-four women (mean age: 26, 19-41) whose mothers experienced severe psychosocial stress in form of an objective life event during their pregnancies and 27 female age- matched controls were exposed to a psychosocial stressor (TSST, Trier Social Stress Test). To control for postnatal environment, all subjects filled out the maternal care scale of the Parental Bonding Index. Maternal care values did not differ between prenatally stressed subjects and controls. Differential blood counts and analysis of lymphocyte subpopulations by flow cytometry before and after the TSST were performed. Total number of lymphocytes and the following lymphocyte subpopulations were assessed: CD3+ (total number of T cells), CD3-/CD19+ (B cells), CD3+/CD4+ (T-helper cells), CD3+CD8+ (T-suppressor/ cytotoxic T cells), CD3+/CD16+, 56+ (Natural killer (NK) cells). **Results**: Blood counts of all immune cells changed significantly after the TSST. While total number of lymphocytes, T cells, T-suppressor cells, B cells and NK cells increased after TSST exposure, the number of T-helper cells decreased after the stress test. There was a significant group x time interaction for total number of lymphocytes (F=4.38, p<.05) and T cells (F=4.59, p <.05) with prenatally stressed subjects showing lower numbers of lymphocytes and T cells before but higher numbers after the TSST compared to controls. There was a similar trend for T-suppressor cells (F=3.63, p=.062) whereas numbers of T-helper cells, NK cells and B lymphocytes did not differ between groups before and after the TSST. **Conclusion**: Prenatal stress might program life long alterations of stress sensitive systems, including immune function. These changes may lead to an enhanced responsiveness of distinct parts of the immmune system to psychosocial stress.

O-051

Autonomic Nervous System and Baroreflex Function Differs in Women who were Small at Birth, Increasing their Cardiovascular Responses to Stressors Alexander Jones,[1] Alessandro Beda,[2] Alexandra M.V. Ward,[1] Clive Osmond,[1] Vivienne M. Moore,[3] David M. Simpson,[2] David I.W. Phillips[1]; [1]*MRC Epidemiology Resource Centre, University of Southampton, UK. [2]Institute of Sound and Vibration Research, University of Southampton, UK. [3]Department of Public Health, University of Adelaide, Australia.*

Background: There is increasing evidence from both animal and human studies that adverse prenatal environments lead to the birth of offspring that have elevated neuroendocrine responses to novelty and aversive stimuli. We recently demonstrated that restricted fetal growth is associated with exaggerated blood pressure responses to psychological stressors. Exaggerated responses are known to increase the risk of developing sustained hypertension in adult life. Although it is suspected that these blood pressure responses to psychological stressors primarily reflect increased autonomic function, the mechanisms are not well characterized. **Aim**: To determine whether a restrictive fetal environment, reflected by small size at birth, persistently alters autonomic nervous system and baroreflex control of cardiovascular function resulting in exaggerated blood pressure and heart rate responses to stressors. **Methods**: A prospective cohort of South Australian men and women, born during 1975-76, were studied. The participants underwent a series of three standard psychological stressors (the Stroop (colour-word conflict) task, a mirror-tracing task and a public speaking task) whilst their blood pressure and pulse rate were recorded continuously using a

finger blood pressure monitoring device (Portapres). Indices of autonomic and baroreflex function were derived using standard spectral analytical techniques. **Results**: Women who were small at birth demonstrated greater systolic blood pressure during the stress tasks ($r = -0.42$, $P < 0.01$) and greater stress-induced increases in blood pressure ($r = -0.37$, $P < 0.01$) and heart rate ($r = -0.31$, $P < 0.05$) with respect to resting levels. During stress, low birthweight women had increased sympathetic activation, indicated by low frequency systolic arterial pressure variability ($r = -0.41$, $P < 0.001$), and parasympathetic withdrawal, indicated by high frequency heart period variability ($r = 0.22$, $P < 0.05$). They also demonstrated reduced baroreflex sensitivity ($r = 0.34$, $P < 0.001$) during stress. In multiple regression analysis, these findings were independent of potential confounders such as gestational age, obesity, smoking, socioeconomic status and depression. No associations between size at birth and cardiovascular function during stress were found in men. **Conclusions**: We found strong associations between markers of impaired fetal growth and autonomic cardiovascular control, which were restricted to women. This involved modulation of both sympathetic and parasympathetic function. We also provide the first human evidence of a relationship between size at birth and baroreflex function. These findings suggest that the prenatal environment has marked and lasting effects on autonomic and baroreceptor control of cardiovascular function in women, with potential consequences for their risk of hypertension and cardiovascular disease in adulthood.

O-052

The Impact of Maternal Betamethasone Administration on the Feral Ovine Kidney [1]Amanda J. Meyer, [2]Karen M. Moritz, [1]Deborah M. Sloboda, [1]Timothy J. M. Moss, [3]Brendan J. Waddell and [1]John P. Newnham: *[1]School of Women's and Infants' Health, The University of Western Australia, Perth, Western Australia, Australia.* [2]Department of Anatomy and Cell Biology, Monash University, Melbourne, Victoria Australia; [3]School of Anatomy and Human Biology, The University of Western Australia, Perth, Western Australia, Australia.

Background: Increased prenatal glucocorticoid exposure in early gestation alters the expression of various glucocorticoid-sensitive genes in the fetal sheep kidney (Hantzis *et al.*, 2002; Moritz *et al.*. 2002). Women at risk of pre-term delivery between 24 and 34 weeks' gestation receive synthetic glucocorticoids to enhance fetal organ maturation. The aim of the present study was to examine the effect of late-gestation maternal betamethasone administration on the expression of glucocorticoid-sensitive genes in fetal sheep kidney. **Methods:** Pregnant ewes (n=44) bearing single male fetuses were injected intra-muscularly (i.m.) with 150 mg of medroxyprogesterone acetate at 100 days of pregnancy (d) to prevent glucocorticoid-induced pregnancy loss. Ewes were then randomized into treatment groups and received i.m. injections of either betamethasone (0.5mg/kg ewe body weight) or saline , on 104, 111, and 118d. Fetal tissues were collected at 109, 116, 121, and 146d. Total protein and ribonucleic acid (RNA) were extracted from the right fetal kidney. Glucocorticoid receptor (GR) and sodium/potassium-adenosine triphosphatase-alpha 1 (Na^+/K^+-ATPase-a^1)protein levels were measured by Western blotting. The relative expression levels of GR, mineralocorticoid receptor (MR), Na^+/K^+-ATPase-a^1, angiotensin II type 1 receptor (AT_1R), angiotensin II type 2 receptor (AT_2R) and angiotensinogen (A_0) messenger RNA (mRNA) were measured using real-time reverse transcriptase-polymerase chain reaction. Data from control and treated animals were compared at each gestational age using *t*-tests, and across gestation using two-way ANOVA with Holm-Sidak post-hoc analysis. **Results:** Maternal betamethasone administration did not affect the relative expression of fetal renal GR, MR, Na^+/K^+-ATPase-a^1, A_0, AT_1R, or AT_2R mRNA at any age. Further, fetal renal GR protein levels at 109, 116, 121, and 146d were unaltered by betamethasone exposure. Renal Na^+/K^+-ATPase-a^1 protein levels at 116d were transiently increased by betamethasone exposure (saline:108± 21, betamethasone: 170± 13; arbitrary optical density units: mean ± S.E.M.; p=0.03) but values at 109, 121, and 146d were not different between groups. Renal AT_2R mRNA levels in both groups were lower ($p<0.001$) at 146d (0.42± 0.11; arbitrary expression units) than at other times (109d: 0.95± 0.09; 116d: 0.98± 0.11; 121d: 0.77± 0.15). In contrast, Na^+/K^+-ATPase-a^1 mRNA levels were higher ($p<0.01$) at 146d (2.12± 0.33) than at other ages (109d: 0.98± 0.03; 116d: 0.91± 0.06; 121d: 1.10± 0.07). **Conclusions:** The increase in Na^+/K^+-ATPase-a^1 mRNA and the reduction in AT_2R mRNA in late gestation are consistent with previous findings. AT_2R expression declines after the completion of nephrogenesis at 130d. The increase in fetal plasma cortisol levels in late gestation is known to augment renal Na^+/K^+-ATPase-a^1 activity in order to increase sodium reabsorption. Increased Na^+/K^+-ATPase-a^1 protein at 116d may be associated with the transient elevation in fetal plasma cortisol levels at this time (data not shown). Fetal renal GR protein levels were unaltered by betamethasone exposure. Maternal betamethasone administration did not alter the renal expression of genes involved in glucocorticoid hormone action or components of the renin0angiotensis system at any gestational age. In conclusion, these data suggest that maternal betamethasone administration in late gestation does not program permanent changes in the renal expression of various glucocorticoid-sensitive genes or proteins in fetal sheep. HANTZIS, V. *et al.*, (2002) *Kidney Intl* **61**, 405-413; MORITZ, K. M. *et al.*, (2002) *Endocrinology* **143**, 4455-4463.

O-053

A Developmental Perspective of Immune and Nociception Functioning Following Prenatal Exposure to Bacteria Nicolette A. Hodyl, Klara M. Krivanek and Deborah M. Hodgson; *Laboratory of Neuroimmunology, School of Behavioural Sciences, University of Newcastle, NSW 2308, Australia.*

Background: Exposure to infection during pregnancy is known to be a major factor in predicting future health outcomes for the offspring. In previous studies, we have demonstrated that exposure to an immune stimulus during pregnancy results in altered immune and hypothalamic-pituitary-adrenal (HPA) axis function in the adult offspring. Given that recent evidence has identified a role for immune factors (e.g. cytokines) in the perception of pain, the current study sought to determine whether the perception of pain was also altered following prenatal exposure to bacterial endotoxin. Additionally, the current study examined whether altered immune and HPA function were also evident in the offspring at different developmental time-points. More specifically, we examined the corticosterone, cytokine and nociceptive response to an immune stimulus in the offspring in the neonatal period (age 5 days), pre-weaning (19 days), adolescent (50 days) and in the adult (90 days) following maternal exposure to endotoxin during gestation. **Methods**: Pregnant Fischer 344 rats were administered either endotoxin (Salmonella Enteritidis, 200ug/kg, s.c.) or saline (equivolume) on gestational days 16, 18 and 20. On postnatal days (PND) 5, 19, 50 and 90 offspring from each prenatal treatment group were administered endotoxin (Salmonella Enteritidis, 50ug/kg i.p. (PND15 and 19) or 100ug/kg i.p. (PND 50 and 90)). Animals were decapitated and blood collected at either baseline or 4 hours post injection, to analyse serum concentrations of corticosterone, interleukin-1beta (IL-1ß) and tumour necrosis factor alpha (TNFa). Nociceptive thresholds were assessed on PND 90 using spinal and supra spinal assessment methods - the hot-plate, tail flick and electro von-Frey tests. **Results**: The corticosterone response to the endotoxin challenge was dependant on the developmental time point tested. More specifically, at both ages 5 and 19 days, the offspring of endotoxin treated dams produced significantly less corticosterone than the control offspring ($p<.05$) in response to immune stimulation. Interestingly, the direction of this response had reversed by the time these animals reached adulthood, with these same offspring producing higher amounts of corticosterone compared to the control offspring at this time point. While the TNFa response to the endotoxin was significantly attenuated in offspring from prenatal endotoxin treated mothers compared to control offspring ($p<.05$), a concurrent reduction in IL-1ß levels was not evident at any of the assessed time-points. Pain responsivity was altered in the offspring of dams treated prenatally with endotoxin, however this effect was specific to the nociceptive test. Endotoxin treated offspring were significantly more sensitive to pain four hours following immune stimulation compared to control offspring ($p<.05$), on tests of both spinal pain (i.e. tail flick) and supra-spinal pain (i.e. hot plate) pathways. While the control offspring showed an analgesic response on the von-Frey test following immune stimulation, this analgesic response was not apparent in the offspring of endotoxin treated dams ($p<.05$). Additionally, sex-specific differences were observed in nociceptive responses. **Conclusions:** Prenatal exposure to endotoxin results in permanent alterations to HPA function, TNFα, IL-1β and nociceptive responses to immune stimulation throughout life. Both HPA and immune factors can impact on nociception. While it is not apparent whether the ontogeny of these systems was independently altered following prenatal bacterial exposure, or if altered HPA / immune function caused altered pain perception, it is nonetheless apparent that shifts in the internal milieu of the prenatal environment can result in permanent alterations to an array of physiological consequences that are significantly related to health outcomes.

Parallel Session 4B: CVD and Risk Factors

O-054

Food Restriction During Gestation, Weight at Birth and Offspring Blood Pressure at Age 59 y: The Dutch Famine of 1944-45 Aryeh D. Stein; Karin M. van der Pal – de Bruin; Patricia A. Zybert; L.H. Lumey. Dept. of Global Health, Emory University, Atlanta GA, USA; TNO Quality of Life, Leiden, Netherlands; Dept. of Epidemiology, Columbia University, New York NY, USA

Background: Few studies in humans have assessed the role of maternal nutrition in the inverse association between offspring birth weight and later blood pressure (BP). We used the circumstances of the Dutch Famine of 1944-45, during which official rations were <900 kcal/day for 24 weeks, to assess whether generalized reductions of maternal food intakes at specified stages of pregnancy were related to offspring systolic (SBP) or diastolic (DBP) BP or hypertension. **Methods**: We recruited three series of subjects: (1) individuals born in one of 3 institutions in western Holland between January 1945 and March 1946, whose mothers were all exposed to the famine during or immediately preceding pregnancy; (2) individuals born in the same 3 institutions during 1943 or 1947, whose mothers had no famine exposure during this pregnancy; and (3) a same-sex sibling of subjects in series 1 or 2. Blood pressure was measured during clinical examinations (total n=971) conducted between 2003 and 2005. We considered as hypertensive those individuals with a prior diagnosis of hypertension or with measured SBP or DBP above 140 / 90 mmHg. We defined four (partially overlapping) periods of gestational exposure (gestational weeks (GW) 1-10; 11-20; 21-30; and 31 through delivery) based on exposure to a ration <900 kcal/day during the whole 10-week interval. Maternal preconception depletion was characterized by a score representing cumulative weeks of exposure to reduced rations in the 6 mo prior to conception. We used multiple linear regression to assess the joint effects of exposure in one or more periods of gestation, implemented in a hierarchical approach (GEE) to account for

within-sibship correlations. We adjusted initially for age and sex, and subsequently for height, waist circumference, alcohol and tobacco use, and prior diagnosis and treatment of hypertension. We tested for heterogeneity of estimates by sex. **Results**: SBP and DBP were 140.3 (mean) +/-20.3 (S.D.) mmHg, and 85.8+/-11.0 mmHg, respectively, with 61.4% prevalence of hypertension. Exposure to reduced rations prior to conception or in any 10-week GW was not associated with SBP level or hypertension prevalence ($p>0.10$ for all tests). For DBP, only exposure to reduced rations in GW 21-30 was associated with an increase of 2.18 mmHg (95% confidence interval (CI) 0.08, 4.29; $p<0.05$) in DBP; covariate adjustment attenuated this association. No other periods of famine exposure had significant associations with DBP. In sex- and age-adjusted models, birth weight was inversely related to SBP (4.13 mmHg increase per 1 kg decrease in birth weight; $p<0.01$), DBP (2.10 mmHg increase per 1 kg decrease in birth weight; $p<0.05$) and the prevalence of hypertension (odds ratio 0.67 per kg birth weight, 95% confidence interval 0.48 – 0.93, $p<0.02$). The estimates for SBP and DBP were attenuated by further covariate adjustment; the estimate for hypertension was robust to adjustment. Adjustment for period of famine exposure did not alter the coefficients for birth weight. **Conclusions**: In this population famine exposure was not associated with later blood pressure and the inverse association of birth weight with SBP and DBP was independent of effects of famine exposure on birth weight. These results suggest that the association between birth weight and later blood pressure does not result from effects of acute maternal undernutrition, but likely relates to other aspects of the pregnancy and placental-fetal nutrition.

O-055

Reduced Nephron Endowment and Hypertension Emerge Following Placental Restriction in the Rat Mary E. Wlodek[1], Julie A. Owens[2], Andrew L. Siebel[1] and Karen Moritz[3]. [1]*Department of Physiology, University of Melbourne, Parkville, Victoria, 3010;* [2]*Gynaecology and Obstetrics, University of Adelaide, Adelaide, 5005 and* [3]*Anatomy and Cell Biology, Monash University, Victoria, 3800 Australia.*

Background: Fetal growth restriction followed by accelerated growth in infancy and childhood is implicated as contributing to the increased risk of developing adult hypertension. In Western society, impaired uteroplacental blood flow is the major characteristic of human pregnancies complicated by intrauterine growth restriction. We have shown that placental restriction in the rat impairs fetal growth and mammary function and pup milk intake after birth. It has been hypothesized that nephron number may be a major determinant of adult blood pressure and the renal renin angiotensin system (RAS) is affected in many models of fetal programming. We therefore determined the effects of placental restriction and the pattern of postnatal growth on adult blood pressure, nephron endowment and renal AT1 receptor mRNA expression. **Methods:** Bilateral uterine vessel ligation (Restriction) or sham surgery (Control) was performed on day 18 of gestation in Wistar Kyoto rats (approved University of Melbourne Animal Ethics Committee). Pup weights were measured from 3 days (d) to 22 weeks (wk) of age. Tail-cuff blood pressure was measured at 5, 9, 14 and 22 wk. Glomerular number and size was estimated using unbiased stereology in 6 month (mo) old males. Renal gene expression was quantified using real-time PCR in 6 mo males and females. Data were analysed by unpaired t-test. **Results:** At 3 d after birth, Restricted male pup weight was 73% and that of females 77% of Controls. Restricted female pups caught up in weight to Controls during lactation ($p<0.05$), whereas Restricted male pups did not ($p<0.05$). Restricted male and female pups demonstrated accelerated growth between wk 5 and 10 and their weights were similar to Controls at 10 wk. However, by 22 wk, Restricted male and female pups were lighter than Controls ($p<0.05$). Restricted males, but not females, had elevated blood pressure compared to Controls (+9 mmHg, $p<0.05$) at 22 wk. At 6 mo, significant left ventricular hypertrophy was detected in Restricted males (75.0±2.6% of total heart weight) compared to Controls (67.7±2.1% of total heart weight) ($p<0.05$), while their kidney weight relative to body weight was unchanged. At 6 mo, Restricted male offspring tended to have fewer nephrons (-18%) with significant glomerular hypertrophy (+31%, $p<0.02$), resulting in normal total glomerular volume compared with Controls. Renal AT1a receptor mRNA expression tended to be higher in Restricted males (+32%, $p=0.07$), but not females, compared to Controls, while renal AT1b receptor mRNA expression was unchanged at this age. The level of relative renal AT1a receptor mRNA expression in females was twice that of males in Control groups ($p<0.02$). **Conclusions:** Placental restriction reduced size at birth and altered postnatal growth in both sexes, with early catch-up in females but not males during lactation. Following placental restriction, only male offspring developed hypertension, left ventricular hypertrophy and glomerular hypertrophy, suggesting multiple and sex-specific pathways to the development of adult hypertension. The nutrition provided to growth-restricted infants both during lactation and after weaning may be an important factor in these consequences for cardiovascular function, as well as growth. The tendency for lower nephron endowment and the increase in renal AT1a mRNA expression is observed following other prenatal challenges, suggesting alterations in renal development and renal RAS may be a common mechanism leading to adult hypertension.

O-056

The Inverse Association of Birth Weight with Systolic Blood Pressure in Adolescence and Adulthood: Meta-Analysis of 20 Nordic Studies *Michael Gamborg on behalf of The Nordic Longitudinal Epidemiological Research Program "Prenatal and Childhood Growth in Relation to Cardiovascular Disease. Institute of Preventive Medicine, Copenhagen University Hospital, Denmark.*

Background: Birth weight (BW) has been associated with subsequent systolic blood pressure (SBP), in a large number of studies. It has been argued, however, that this association is a statistical artefact due to adjustment for current body mass index (BMI). The purposes of this study are to investigate if BW is associated with SBP even without adjustment for current BMI and to examine the shape and the heterogeneity of the association by sex and age. **Populations:** Data from 20 cohorts in 6 Nordic countries were included in this study. These cohorts differed with respect to year of birth, sex, and age at blood pressure measurement. Overall, 200,871 subjects had information available for BW, BMI and SBP. **Methods:** To investigate the association between BW and SBP, we performed meta-regression analyses. However, as we had access to original data, we used a novel approach. Firstly, the researchers responsible for each cohort performed regression analyses stratified by sex and age and adjusted and unadjusted for current BMI. Secondly, the stratum specific estimates, along with the corresponding standard errors, were reported to the coordinating centre. Thirdly, the coordinating centre pooled the stratum specific estimates, using random-effects meta-regression. **Results:** We detect an inverse association between BW and SBP in later life, both in models adjusted and unadjusted for current BMI. The associations are stronger in the models that include current BMI. Among men there is evidence of linearity. Among women with a BW ≤4kg, there is an inverse association between BW and SBP, however for BW >4kg the association changes direction and becomes positive, even when adjusting for current BMI. The strength of the inverse association is stronger in the older cohorts compared to the younger cohorts. For example in a model unadjusted for BMI, the estimated change in SBP per kg increase in BW is -1.24 mmHg (95% CI: -2.01 to -0.46) among 30-year-old males and -1.79 mmHg (95% CI: -3.21 to -0.37) among 50-year-old males. Among women with a BW ≤4 kg, the estimated change in SBP per kg increase in BW is -1.46 mmHg (95% CI: -2.36 to -0.56) among 30-year-olds and -1.96 mmHg (95% CI: -4.41 to 0.48) among 50-year-olds. **Conclusion:** In this study, we find that the inverse association between BW and SBP is present even without adjustment for current BMI, which suggests that it is not just a statistical artefact that arises when current BMI is included in the model. The shape of the association differs between men and women, with non-linearity evident for women with a birth weight >4kg. This suggests that the mechanisms underlying the association are sex specific. Further, the association becomes stronger with age in both men and women. A potential explanation is that SBP is measured in mmHg, and the distribution of SBP is wider in older populations. This implies that the difference between a normal SBP and a high SBP becomes larger with age.

O-057

Maternal Iron Deficiency During Pregnancy in the Rat Induces High Blood Pressure, Obesity and Dyslipidaemia in Her Offspring Lorraine Gambling, Christopher A Maloney, Henriette S Andersen & Harry J McArdle. *Rowett Research Institute, Aberdeen UK.*

Background: Iron deficiency is a common and serious nutritional disorder, with well-known and serious sequelae. If it occurs during pregnancy, these continue into the adult offspring. We have developed a rat model of maternal iron (Fe) deficiency and show that offspring born to Fe deficient mothers developed high blood pressure in postnatal life (1). Here, we expand our studies on maternal Fe deficiency during pregnancy and the long-term health of the offspring. **Methods:** Female Hooded Lister rats (n=20) were fed a control diet for 2 weeks from weaning. The females were then fed diet with control (50 mg/kg; C) or deficient (7.5 mg/kg; D) Fe content for four weeks prior to mating and throughout pregnancy. At birth, litters were culled to 8 and the pups cross-fostered to control fed dams. All male pups were weaned onto control diet. Blood pressure was measured by the tail cuff method. Body composition was analysed using an EchoMRI analyser. Glucose tolerance test was performed at 35 weeks. Blood and tissue samples were collected at 38 weeks. Data are mean ± s.e.m., n=18 litters, significance was by unpaired Students t-test. **Results:** Consistent with our previous studies (1, 2), at birth pups from deficient dams were significantly smaller (C, 5.9±0.1; D, 5.2±0.1g, $p=0.001$). Until 14 weeks of age the offspring born to Fe deficient dams continued to be smaller (Area under curve, $p=0.03$). A significant increase in growth rate in the offspring of Fe deficient dams from 14 to 18 weeks (C, 1.1±0.1; D, 1.5±0.1 g/day, $p=0.003$) led to there being no significant difference in body weight between the control and experimental offspring from 14 weeks to the end of the experiment at 38 weeks of age. As shown previously male offspring from Fe deficient dams had raised blood pressure compared to their control counterparts at 6 and 16 weeks. In this study blood pressure in the offspring was measured at 35 weeks and again those from Fe deficient dams had raised blood pressure (C, 154±4; D, 166±3 mmHg, $p=0.03$). At 10 weeks of age there was no difference between the two groups in body composition. By 16 weeks of age lean body mass was decreased, fat mass and fat/lean mass ratio increased in the offspring from Fe deficient dams. A progression in the effect on body mass composition was seen, with the differences becoming greater at 24 and 35 weeks (Figure). Serum triglycerides (C, 1.32±0.08; D, 1.53±0.07 mmol/l, $p=0.05$) and cholesterol (C, 2.51±0.07; D, 2.94±0.16 mmol/l, $p=0.03$) levels are increased in the offspring from Fe deficient dams compared to controls at 38 weeks of age. There was no significant difference between the two groups of offspring in either glucose or insulin levels following a glucose challenge at 36 weeks of age. **Conclusion**: These data demonstrate that the increase in blood pressure occurs before the onset of obesity and not as a result of obesity. By cross fostering the pups, we have taken out confounding factors, such as maternal Fe deficiency during lactation and show unequivocally, that prenatal Fe deficiency is a causal factor in the development of adult disease in the offspring. **References:**1. Gambling *et al.* (2003) *J. Physiol.* 552: 603-610. 2. Gambling *et al.* (2004) *J. Physiol.* 561: 195-203

O-058

Against Programming Patrick Bateson, *Sub-Department of Animal Behaviour, University of Cambridge, High Street, Madingley, Cambridge CB3 8AA, UK*

Different adaptive phenotypes arising from a common genotype are reported with ever increasing frequency. In these examples of developmental plasticity an environmental condition or maternal state commonly establishes a particular trajectory of development in early life. Unfortunately, the terminology for these processes has become confused. To call them "programming" is somewhat similar to saying that pressing a button on a juke box is "recording" the tune. Misleading metaphors in science matter if they confuse or send research off in unprofitable directions. Some signs suggest that this has already happened with programming. A reputable and long-established term from developmental biology that would do instead is "induce" but a perfectly good neutral word would be "elicit" which might produce a graded or a discrete response. With this confusion cleared up, a distinction could then be drawn between developmental process and developmental outcome. The outcome could be described, if you must, as "programmed by Darwinian evolution" but not by the conditions of early life. Could we use this conference to clean up our act?

O-059

Prenatal Restraint Stress and Behavior in Fischer 344 Rats; The Implications of a Subsequent Exposure to Stress Daniël L.A. Van den Hove*/**, Harry W.M. Steinbusch**, Matteo Bruschettini*/**, Hellen Steinbusch*/**, Jos Prickaerts**, Carlos E. Blanco*, *Pediatrics/GROW, **Psychiatry and Neuropsychology/Brain and Behavior (EURON), Maastricht University, 6200 MD Maastricht, Netherlands.

Background: In recent years, there has been increasing awareness that chronic exposure to stress during prenatal development can predispose progeny to various psychological disorders in adulthood. However, recent findings in Fischer 344 rats suggested that an adaptive or protective effect of prenatal stress (PS) towards stress should not be excluded. **Objective**: Our main objective was to examine the effect of a subsequent stress exposure on anxiety and depressive-like behavior in PS and control rats. **Design/Methods**: During the last week of gestation (E14-E21) pregnant female Fischer 344 rats were individually restrained in transparent plastic cylinders (for three 45 min periods per day), while, in addition, being exposed to bright light. Control dams were left undisturbed. To examine the effect of a subsequent exposure to stress male PS and control offspring were exposed to a 3-day period of stress (mouse cage/restraint stress/wet bedding) at an age of 3 months. Anxiety and depressive-like behavior of the rats was tested both before and at an age of 6 months. In addition, stress-induced plasma corticosterone secretion and cell proliferation within the hippocampal dentate gyrus (DG) was studied. **Results**: We found PS animals to weight less at birth as compared to control offspring. Further, PS rats were more anxious before the subsequent exposure to stress, as measured in the open field and home cage emergence tasks, whereas, afterwards, they performed relatively better, i.e. they were less anxious, as compared to controls in these tasks. In addition, PS animals initially exhibited more depressive-like behavior, as measured in the forced swim test, which normalized after the subsequent exposure to stress. No differences were observed in stress-induced plasma corticosterone secretion and cell proliferation within the DG. **Discussion**: Our data are in support of 'predictive adaptive response' (PAR) hypothesis, which predicts that PS allows offspring to better cope with stress in later life as compared to offspring that developed under 'normal' prenatal conditions. In conclusion, PS Fischer 344 rats seem to perform relatively better under stressful conditions as compared to control rats. The present data provide further evidence for the idea that PS may also have, dependent upon the genetic background and history, adaptive and/or protective properties. Finally, this study once more underlines the importance of the choice of strain in stress-related investigations.

Parallel Session 4D: Developmental Disruption

O-060

Traffic Polycyclic Aromatic Hydrocarbons (PAHs) Genetic Susceptibility and Risk of Breast Cancer Jing Nie, Jan Beyea, Matthew Bonner, Daikwon Han, John Vena, Peter Rogerson, Dominica Vito, Paola Muti, Maurizio Trevisan, Peter Shields, and Jo Freudenheim; Department of Social and Preventive Medicine, State University of New York at Buffalo, Buffalo, NY, Consulting in the Public Interest, Lambertville, NJ, University of South Carolina, Columbia, SC, Georgetown University, Washington, DC, USA

Background: Growing evidence suggests that there may be critical time periods of exposure in breast cancer initiation and development. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous and exist in the ambient environment at low levels. We previously found evidence that exposure to PAHs based on an estimate of exposure to traffic emissions in a woman's earlier life may be associated with breast cancer risk in adulthood. Glutathione S-transferase mu, a phase II enzyme, is involved in the detoxification of PAHs. There is a common *GSTM1* genetic polymorphism that is a deletion of the entire gene. The *GSTM1* null genotype is associated with a deficient detoxifying enzyme activity. In this study, we examined the association between *GSTM1* genotypes and breast cancer risk, and interaction with traffic emission-PAH exposure estimated for each woman at menarche, at the time when she had her first birth, and at 20 and 10 years prior to interview, using data collected from the Western New York Exposures and Breast Cancer (WEB) study, a population-based case control study. **Methods**: All participants were women, aged 35-79, residents of Erie and Niagara Counties. Cases had incident, primary, histologically-confirmed breast cancer. Controls were randomly selected and frequency-matched to cases on age, race and county. In-person interviews were used to collect data on potential breast cancer risk factors including self-reported lifetime residential history. Blood samples were collected at the time of the interview and used to determine *GSTM1* genotype. A geographic model was used to reconstruct historical traffic PAH exposure at each residence. **Results**: There was no main effect of *GSTM1* on breast cancer risk. While we had previously found an association between higher exposure to traffic emission PAHs and breast cancer risk, we now found evidence that the association was limited to women with *GSTM1* null genotype. For exposure at menarche, limited to women living within 250 meters of a road with traffic counts, the upper quartile of PAH exposure was associated with increased risk of premenopausal breast cancer (OR 4.64, 95% CI 0.98-21.94; p for trend 0.01) and emissions at the time of a woman's first birth was associated with increased risk of postmenopausal breast cancer (OR 3.27, 95% CI 0.99-10.84, p for trend 0.02). There was no association of traffic emissions with risk among women with *GSTM1* wild-type, or for any of the other time periods. **Conclusions**: Our findings suggest that there is increased risk of breast cancer associated with exposure to traffic emission PAHs in early life, and that the association is limited to women with *GSTM1* null genotype.

O-061

Maternal Alcohol Ingestion during Pregnancy Predisposes to Smaller Kidneys and Albuminuria in Aboriginal Children: Findings from an Aboriginal Birth Cohort Gurmeet R. Singh[1], Susan M. Sayers[1] and Wendy E. Hoy[2]; [1] *Public Health and Chronic Disease Division, Menzies School of Health Research and Charles Darwin University, Darwin, Australia.* [2] *Centre For Chronic Disease, University Of Queensland, Brisbane, Australia*

Aim: To examine the relationship of maternal smoking and alcohol use during pregnancy to kidney size and function in c0hildhood, in birth cohort of Australian Aboriginal children. **Background:** It has been proposed that intra-uterine growth retardation causes impaired nephrogenesis resulting in lower nephron numbers and smaller kidneys. This effect could be mediated either directly or via exposure to distal factors such as poor maternal nutrition and/or exposure to alcohol and nicotine during critical periods of renal development. **Methods:** A longitudinal prospective study of an Australian Aboriginal birth cohort (n=686) established between 1987 and 1990 formed the study population. Post-natal maternal weight and height and self reported smoking and alcohol use, and birth size was recorded at the time of birth. Clinical gestational age was assessed within 4 days of birth using the Dubowitz method as last menstrual period (6.5 %) and early sonograms for dating of pregnancy (8.2 %) were rarely available. Wave 2 follow-up at age 8-14 years (mean age 11.5 years) on 572 children included an assessment of kidney function using urine albumin creatinine ratios (ACR; n=533) and an estimation of renal size by ultrasound measurements(n=529). For analysis, kidney volumes corrected for current body surface area (BSA) were divided into quartiles. **Results**: In this cohort, 56% (375/667) of the mothers smoked and 13.6% (88/649) consumed alcohol during pregnancy. Mothers who smoked were more likely to have for small-for-gestational age babies (SGA; OR 1.5, $p < 0.03$). Alcohol use during pregnancy was not associated with either SGA or preterm babies. Antenatal alcohol exposure was associated with a significantly higher proportion of children with pathological albuminuria (= 3.4) adjusted for age, sex and weight; 14.5% compared to 6.5% (p=0.03). Corrected kidney volumes in the lowest quartile were more common in those exposed to alcohol in utero (40% compared to 22%: p=0.003), and the association persisted after adjustment for the effect of SGA, maternal smoking and maternal BMI. Stratification of results by in utero exposure to smoking alone, alcohol alone or exposure to both revealed the same trends, although the numbers of mothers who consumed alcohol but did not smoke were small. **Conclusion**: Alcohol exposure in utero has an adverse effect on renal size and function during childhood. Alcohol consumption during pregnancy may be one of the contributors to the multi-determinant renal disease currently present in epidemic proportions Australian Aboriginal people living in the Northern Territory of Australia.

O-062

Neonatal Exposure to the Phytoestrogen Genistein Adversely Affects Fertilization Rate and Oocyte Quality Later in Life Wendy N. Jefferson[1,3], Elizabeth Padilla-Banks[1], Eugenia H. Goulding[2], E.M. Eddy[2] and Retha R. Newbold[1]; [1]*Developmental Endocrinology Section, Laboratory of Molecular Toxicology, and* [2]*Gamete Biology Section, Laboratory of Reproductive and Developmental Toxicology, NIEHS, NIH, DHHS, RTP, NC 27709 and* [3]*Department of Environmental and Molecular Toxicology North Carolina State University, Raleigh, NC 27605*

Background: Exposure of the developing organism to estrogenic substances is known to cause deleterious effects on the reproductive tract. Previous studies have shown that neonatal exposure to the naturally occurring phytoestrogen, genistein (Gen) causes adverse consequences on the developing female reproductive system. Gen alters ovarian development and function as well as causes infertility and uterine cancer later in life. We have shown that Gen alters ovarian differentiation by preventing the breakdown of oocyte nests during the first week of life, thus resulting in multi-oocyte follicles (MOFs); these effects are mediated through estrogen receptor (ER) ß. To

further study the mechanisms involved in female subfertility and infertility following neonatal Gen exposure, we examined the effects on oocyte quality in culture. **Methods:** Female CD-1 mice were treated with Gen at doses of 5, 25 or 50 mg/kg on days 1-5 or left untreated as controls. At 8 weeks of age, mice were treated with PMSG followed by hCG and then bred to proven control males. The following morning, reproductive tracts were collected from vaginal plug positive mice and placed in D-MEM medium. Oocytes were carefully collected, counted and placed in KSOM+AA medium in a 5% CO2 incubator. The percentage of fertilized oocytes was determined in the afternoon of collection by the appearance of the second polar body. The fertilized oocytes were then followed over the next 4 days to determine progression to the following developmental stages: 2-cell, 4-cell, morula and blastocyst. **Results:** From three separate experiments, the average percent of fertilized oocytes from mice treated neonatally with Gen 50 mg/kg was 43% compared to 86% in controls. Most of the fertilized oocytes in both treatment groups progressed to the 2-cell stage (controls, 100%; genistein, 89%) and the 4-cell stage (control, 93%; genistein, 70%). However, there was a statistically significant decrease in the percent reaching the morula stage compared to controls (control, 72%; Gen, 22%); this difference was even more dramatic at the blastocyst stage (control, 49%; Gen, 9%). To determine if these alterations are seen at lower doses, an additional experiment was performed. The percent of fertilized oocytes from mice treated with Gen-5 and Gen-25 was 90% and 80%, respectively, compared to 86% in the controls. The percentage of fertilized oocytes that reached the blastocyst stage was 31% in the Gen-5 treatment group and only 3% in the Gen-25 treatment group compared to 49% in controls. **Conclusion:** Taken together, our data supports the idea that brief exposure to environmentally relevant doses of Gen, found in soy products including soy based infant formulas, is associated with altered ovarian differentiation, lower fertilization rates and poor oocyte quality.

O-063

Lead (Pb) and the Developmental Origin of Alzheimer's Disease Md. Riyaz Basha[1], Jinfang Wu[1], Wei Wei[1], Hassan Siddiqi[1], Brian Brock[1], Amy Anderson[1], Yuan-Wen Ge[2], Debomoy K. Lahiri[2], Jean Harry[3], Deborah C. Rice[4], and N. H. Zawia[1]

[1]Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI 02881, USA; [2]Laboratory for Molecular Neurogenetics, Department of Psychiatry, Indiana University School of Medicine, Indianapolis, Indiana 46202, USA; [3]Nation Institutes of Health, Research Triangle Park, NC 27709, USA; [4]Maine Department of Health and Human Services, 11 State House Station, Augusta, Maine 04333, USA.

Background: The concept of the Fetal Basis of Adult Diseases (FeBAD) heralded an approach that disturbances occurring at the critical periods of organ development may lead to "programmatic" changes via gene expression or gene imprinting that result in functional deficits later in life. The ability of adverse pregancy outcomes to increase the risk for adult diseases has been amply supported by many epidemilogical studies; however, the molecular mechanisms that mediate such long-term or delayed effects and the potential for environmental exposures to alter the course of adult disease has not been demosntatred. Alzheimer's Disease (AD) is a progressive neurodegenerative disorder found in elderly populations and the predominantly sporadic nature of AD suggests a role for the environment in the onset of this disease. To examine the link between developemntal exposures and diseases of old age, we have studied the relationship between lead (Pb) expsoure and pathogenesis associated with AD using rodent and monkey models. Recently we have reported that developmental exposure (birth through weaning) to Pb resulted a delayed (20 months later) over-expression of amyloid precursor protein (APP) mRNA and protein and its amyloidogenic Aβ product in rats. In contrast, APP expression and Aβ levels were unaltered by Pb-exposure during old age (18-20 months of age). In order to validate our studies on rodents, we acquired brain samples of 23 year old Cynomolgus monkeys with a similar Pb-exposure scenario, and examined the molecular and pathological consequences associated with AD. **Methods:** ***Pb-Exposure:*** Cynomolgus monkeys were exposed to 0 or 1.5mg/kg/day Pb (lead acetate) from birth through 400 days of age. Animals were sacrificed at 23 years of age and various brain regions were isolated and stored at -80°C. ***APP & Aβ:*** APP mRNA APP and Aß levels were estimated in the control and Pb-exposed monkey cortices. mRNA was determined using RT-PCR technique. APP levels were monitored by Western Blot analysis and the levels of Aß were measured by ELISA, using human IBL-America kits. ***Immunohistochemistry:*** Monkey brains (perfused and postfixed) were sectioned and subjected to DAB Labeling using specific primary antibodies for APP-N, SP1, Aß. **Results:** Consistent with our preceding rodent results, monkeys exposed to Pb as infants exhibited an enhancement in the characteristic features of AD such as significant ($p<0.05$) increase in the levels of APP mRNA, APP, $A\beta_{1-40}$ and $A\beta_{1-42}$. Interestingly the immunohistochemical analysis using Aβ staining showed the presence of abundant plaques in the Pb-exposed monkey cortices. **Conclusions:** This new body of research presents compelling evidence that developmental exposure to environmental agents even at low levels results in delayed consequences on health and may manifest disease in old age. These findings further suggest that exposure to environmental agents during brain development predetermine the expression and regulation of certain genes later in life, thereby altering the course of neurodegeneration.

Parallel Session 4E: Intervention Studies in the Developing World

O-064

Antenatal Micronutrient Intervention Effects on Birth Weight and Infant Mortality Vary by Gender of the Newborn Parul Christian, Subarna K. Khatry, Steven C. LeClerq, Joanne Katz, Sharada R. Shrestha, Keith P. West Jr.; Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA and the Society for Prevention of Blindness, Tripureswor, Kathmandu, Nepal.

Background: Previously we have shown that antenatal micronutrient supplementation in rural Nepal results in an improvement in birth weight and 6-mo infant survival. Here we explore the treatment effects by gender of the newborn to examine effect modification. **Methods**:In Nepal, a community-based, randomized, double-masked trial was conducted to examine effects of daily, antenatal supplementation with four combinations of micronutrients on birth weight and infant mortality. Pregnant women received daily, from early gestation through 3 months postpartum folic acid, folic acid+iron, folic acid+iron+zinc and a multiple micronutrient supplement containing the foregoing plus 11 other micronutrients, all along with vitamin A compared with vitamin A alone as the control group. Birth weight was measured within 72 hours of birth in 80% of the infants. Vital status was monitored for 99% of all live born infants. **Results:** Over the course of a year, 4986 women were enrolled in the study who gave rise to 4130 live births. The male:female ratio at birth was 1.02. Mean birth weight was lower in the control group among females relative to males (2532 vs. 2644 g) as was prevalence of low birth weight (49.4% vs. 37.0%). Overall, maternal supplementation with folic acid+iron and multiple micronutrients increased birth weight by ~60 g. The combinations of folic acid alone and folic acid+iron+zinc had no impact on birth weight. Stratified analysis by gender showed that while the impact of folic acid+iron and multiple micronutrients was higher among female than male infants, the interaction was not statistically significant. Folic acid alone increased birth weight by 30 g (-42, 102g) in females but decreased it to a similar extent in males (-39 g, 95% CI: -110, 32 g); the p-value for interaction was 0.12. Similarly, antenatal folic acid+iron+zinc supplementation showed similar qualitative difference, increasing birth weight among females and decreasing it in males (44 g vs. -34 g, p-value for interaction: 0.07). With regard to 6-mo infant mortality, overall we reported a non-significant reduction of about 20% associated with folic acid, folic acid+iron or folic acid+iron+zinc supplementation. Multiple micronutrient supplementation did not decrease mortality despite increase birth weight. Male 6-mo mortality was higher at 73/1000 live births compared to female mortality (51/1000) in the control group. Stratified analysis by gender revealed no effect modification of treatment for the various combinations of supplements except folic acid+iron+zinc. Supplementation with this combination was associated with a significant reduction in male 6-mo mortality (RR=0.56, 95% CI: 0.85-0.99) but not in females (RR=1.28, 95% CI: 0.74, 2.22); p-value for interaction 0.05. **Conclusion:** In this area of Nepal, the mean birth size of female infants was lower by about 100 g, with the rate of low birth weight being higher (50%) in female than male newborns. In contrast, mortality in the first 6 months of life is higher among males than females. Effects of either folic acid+iron or multiple micronutrients did not differ by gender for either outcome. In contrast folic acid+iron+zinc increased birth weight in females but not males whereas it reduced mortality in males but not females. This suggests that modifications in birth weight and alterations in survival due to maternal micronutrient supplementation may vary by gender and mixture. Further investigation of gender-related differentials in later life function related to early developmental exposures is needed.

O-065

Impact of Maternal Nutritional Supplementation with Respect to Pre-pregnancy Nutritional Status, Maternal Weight Gain and Fetal Growth as Detected by Ultrasound Yukiko Wagatsuma, Lynnette M. Neufeld, Shams El Arifeen, Dewan S. Alam, Edward A. Frongillo, Kathleen M. Rasmussen and Lars Åke Persson. Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Japan; National Institute of Public Health, Cuernavaca, Mexico; ICDDR,B: Centre for Health and Population Research, Dhaka, Bangladesh; International Nutrition, Cornell University, Ithaca, New York, USA; International Maternal and Child Health, Uppsala University, Uppsala, Sweden.

Background: Seventeen million infants are born with low birthweight (LBW) in developing countries in each year. Although LBW has multiple etiologies, intrauterine growth retardation (IUGR) accounts for the great majority of the LBW in developing countries. The purpose of this study is to describe the fetal growth in a population where maternal malnutrition is prevalent, and to determine whether maternal nutritional supplementation introduced during the first trimester of pregnancy had more positive effect on fetal growth assessed by ultrasound compared to the usual later introduction of maternal supplementation. **Methods:** This study was conducted in a rural area of Bangladesh (Matlab) where the health and demographic surveillance system is maintained. All pregnant women were randomized to receive 600 kcal food supplementation beginning in early or late pregnancy and multiple micronutrients or iron and folic acid only. All enrolled women were examined by ultrasound first at 8-13 weeks of gestational age. Various sonographic parameters were measured 3 times during pregnancy. **Results:** Out of 5580 pregnant women identified as eligible for the study, 4436 women were successfully randomized to the early and usual nutritional

treatments at a mean gestational age of 9±2 (SD) week. Fetal growth during the first half of gestation approximated the 50th percentile of reference values except for abdominal circumference, which was significantly ($P < 0.01$) lower. At 31-36 week gestation, head and abdominal circumference as well as femur and tibia length were significantly ($P < 0.05$) below the 50th percentile of reference values. Neither the timing of food supplementation nor type of micronutrient supplement offered to the women in this study affected any measure of fetal growth at any of the 3 times at which it was assessed by ultrasound—except for femur and tibia length with biologically insignificant difference. **Conclusions:** Various IUGR patterns were identified at various timing of gestation. The functional significance of these identified patterns should be evaluated by following the population further to the childhood and adulthood. This study did not provide support to shift from the current strategy of prenatal supplementation promoted by UNICEF and WHO.

O-066

US $ 51 for 100 g Improvement of Birth Weight: What Does it Mean for Food Supplementation Programme of Bangladesh? *Rubina Shaheen[1,2], Shakil Ahmed[2], Lars Åke Persson[3], Zeba Mahmood[4], Lars Lindholm[1]* [1] Epidemiology, Umeå University, SE 901 85, Umeå, Sweden, [2] ICDDR,B: Center for Health and Population Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh [3] International Maternal and Child Health, Department of Women's and Children's Health, Uppsala University, University Hospital, SE 751 85, Uppsala, Sweden, [4]The Micronutrient Initiative, CIDA – PSu, House D2, Road 95, Gulshan, Dhaka 1212, Bangladesh

Background: we have conducted cost-effectiveness (CE) analysis of prenatal food supplementation offered by Bangladesh Integrated Nutrition Project (BINP) and its successor National Nutrition Programme (NNP) in terms of cost for 100 g improvement of birth weight and examined its implications in terms of net social benefit. **Methods:** we used the outcome data from an observational study conducted from December 1998 till October 1999 at Shaharasti under Chandpur district, Bangladesh where only malnourished (Body Mass Index <18.5 kg/m^2) pregnant women were included in the supplementation programme and average about 1 g improvement of birth weight (BW) occurred from each day of supplement. Data on major cost items came from a costing study conducted nearly at the same time (October 1999 till March 2000) when the study at Shaharasti was conducted. Data on additional cost items were collected during the year 2002. All costs were adjusted to represent the present value for the year 2002. Cost per day for supplementing one malnourished pregnant woman was calculated. CE ratio in terms of cost per 100 g improvement of birth weight was calculated by dividing the total cost for supplementing individual woman by the gains in terms of birth weight for individual woman. Gains in terms of birth weight from the original outcome data was used for this purpose. By using the relative risk of 0.47 for low birth weight with 100 kcal daily extra throughout pregnancy from the literature and by using average national income and expenditure also from the literature we examined the meaning of this CE ratio in terms of net social benefit. **Results:** average CE ratio was US $ 50.85 (95% CI US $ 50.79 – 50.90). More favorable CE ratio, US $ 23, was possible for births during January and February, the births that occurred just after the most debilitating months (mid August to mid November). Higher level of adherence to food supplement resulted in more favorable CE ratios among low weight women compared to higher level of adherence to food supplement among higher weight women and for births during January and February compared to births during the other months. With a prevalence of 45% LBW among malnourished women, the average ratio of US $ 50.85/100 g improvement of birth weight would mean a net social benefit of US $ 31 for supplementing one malnourished pregnant woman. **Conclusions:** since increase in birth weight is associated with positive spillover effects even in future generations in addition to positive effects on newborns, for a developing country the amount of economic return from supplementing one malnourished pregnant women seems to be very high and may also be considered such in the global context. Prenatal food supplementation is a worthwhile investment of health resources.

O-067

The Efficacy of Fish Oil Supplementation to Prevent Preterm Delivery

Mina Iravani (MS) *Faculty Of Nursing And Midwifery, Islamic Azad University, Dezful , Iran*

Introduction: Preterm birth is a major cause of perinatal morbidity and mortality. A history of prior preterm delivery strongly correlates with subsequent preterm labor. The purpose of this study was to evaluate the effectiveness of fish oil supplementation to prevent preterm delivery. **Method and Material:** This study was a randomized, double – blind, controlled, clinical trail. Subjects were enrolled in an ambulatory clinic where they received prenatal care. In this clinical trail 120 pregnant women, who had experienced previous preterm delivery , were randomly assigned into two group: the study group (n = 60) receiving 2.54 mg / day fish oil and the control group (n = 60) received placebo in identically – looking capsules from around 20 week until delivery .The groups were compared for preterm delivery and low birth weight . Statistical methods for normally distributed variables included 2- tailed student t test and chi – square analysis. The accepted level of significant was $p < 0.05$. **Results:** The two groups of women did not differ in maternal age gestational age, parity and maternal weight. Following the administration of fish oil , 8 of 60 women (%13.3) began active labor before 37 week compared to 19 of 60 women (%31.6) receiving placebo ($p < 0.01$). Fish oil reduced recurrence risk of preterm delivery from %31.6 to %13.3 in women who had previously experienced a preterm delivery. The mean gestation length was significantly higher in the study group (38.6 week vs 37 week, $p = 0$). Low birth weight occurred in % 16.6 (10 /60) of neonates in the experimental group compared to %35 (21/ 60) in the control group ($p < 0.02$). The mean birth weight of neonate was significantly higher in the study group (2530 gr vs 2400 gr, $p < 0.03$). **Conclusions:** fish oil supplementation reduced the recurrence risk of preterm delivery and low birth weight.

Parallel Session 5A: Infant Feeding / Postnatal Growth

O-068

The Effect of Breast Feeding on Mean Body Mass Index and the Risk of Obesity Across the Lifecourse; A Quantitative Review of Published and Unpublished Observational Evidence

Christopher G Owen, Richard M Martin, Peter H Whincup, George Davey-Smith, Matthew Gillman, and Derek G Cook; Division of Community Health Sciences, St George's, University of London, Cranmer Terrace, London, UK, SW17 0RE. Department of Social Medicine, University of Bristol, Canynge Hall, Whiteladies Road, Clifton, Bristol, UK, BS8 2PR. Department of Ambulatory Care and Prevention, Harvard Medical School and Harvard Pilgrim Health Care, 133 Brookline Avenue, 6th Floor, Boston, MA 02215, USA.

Background: Evidence from observational studies has suggested that breast feeding may reduce the risks of obesity in later life. The purpose of this study was to use both published and unpublished data to examine whether initial breast feeding is related to lower mean body mass index (BMI) and the odds of obesity throughout the lifecourse. **Methods**: A systematic review of published studies investigating the association between infant feeding and a measure of obesity or adiposity in later life, supplemented with data from unpublished sources. Analyses are based on mean differences in BMI and the odds of obesity (mostly based on the 95th or 97th percentile in BMI), amongst those initially breast-fed compared with those formula fed. Mean differences are expressed as breast minus formula fed, odds ratios less than one imply that breast feeding is associated with a lower prevalence of obesity compared to formula feeding. Estimates were pooled using fixed effects models throughout. **Results**: From 70 eligible studies, 36 mean differences in BMI (from 355,301 subjects) and 29 odds ratios (from 298,900 subjects) were obtained. Breast feeding was associated with a small reduction in mean BMI compared with formula feeding (-0.04, 95% CI -0.05 to -0.02 kg/m^2). The mean difference in BMI was smaller, but still apparent, in larger studies of ≥1000 subjects (-0.03, 95% CI -0.05 to -0.02 kg/m^2) and in studies that had not previously published on the association. Adjustment for social status, maternal smoking in pregnancy and maternal BMI, abolished the effect of infant feeding on mean differences in BMI (11 studies, 38445 subjects, -0.10, 95% CI -0.14 to -0.06 kg/m^2 before adjustment; -0.01, 95% CI -0.05 to 0.03 kg/m^2 after). Breast-feeding was associated with a reduced risk of obesity compared with formula feeding (odds ratio 0.87, 95% CI 0.85 to 0.89). The inverse association between breast feeding and obesity was still apparent in larger studies of ≥500 subjects (odds ratio 0.88, 95% CI 0.85 to 0.90). In 6 studies (70744 subjects) that adjusted for all three major potential confounding factors (parental obesity, maternal smoking and social class) the inverse association was markedly attenuated (from an odds ratio of 0.86 to 0.93). Egger tests for small study bias were statistically significant both for mean differences in BMI ($P = 0.002$) and for the odds of obesity ($P < 0.001$). **Conclusion**: Breast fed subjects have a slightly lower mean BMI and a reduced odds of obesity. However, the association between breast feeding and mean levels of BMI may be attributable to confounding and publication bias, though we cannot exclude a modest protective effect on obesity prevalence. Publication of more evidence from studies with well measured confounders will help to resolve these uncertainties.

O-069

Breast Feeding and Risk for Childhood Obesity: Does Maternal Diabetes or Obesity Status Matter?

Elizabeth J. Mayer-Davis, Sheryl L. Rifas-Shiman, Frank Hu, Graham A. Colditz, and Matthew W. Gillman; Center for Research in Nutrition and Health Disparities, Arnold School of Public Health, University of South Carolina, Columbia South Carolina; Harvard Medical School, Harvard Pilgrim Health Care, and Harvard School of Public Health

Background: Previous studies indicate that breast feeding may reduce risk for childhood obesity. However, the impact of breast feeding on subsequent childhood weight may be attenuated among children whose mothers had diabetes, perhaps due to alterations in the nutritional composition of breast milk from diabetic mothers. The purpose of the present analysis was to evaluate whether maternal diabetes (DM) or weight status attenuates a previously reported beneficial effect of breast feeding on childhood obesity. **Methods** Subjects include participants in the Growing Up Today Study (GUTS), who are offspring of women participating in the Nurses' Health Study II. Included were 15,282 girls and boys, age 9 to 14 yrs in 1996. Maternal diabetes and weight status, and infant feeding were by maternal self-report. Maternal overweight was defined as BMI ≥ 25 kg/m^2. Youth self-reported their current height and weight. Childhood obesity was defined based on CDC definitions according to age- and gender-specific BMI as normal (up to 85th percentile), at risk for overweight (85th to 95th percentile), or overweight (>95th percentile). Maternal status was defined categorically as non-DM/normal weight, non-DM/overweight, or DM (regardless of weight). Logistic regression models used generalized estimating equations to account for non-independence between siblings. **Results:** The table shows associations of breast feeding status in the 1st 6 months of life with childhood overweight. Results did

not differ according to maternal diabetes or weight status (p-value for interaction term = 0.29).

	Maternal Status			
Offspring Status	non-DM/Normal Wt n=8617	non-DM/OWT n=6190	DM** n=475	All n=15,282
Percent ever breast fed	89%	86%	86%	88%
Percent overweight	3.5%	11.0%	8.8%	6.7%
	OR (95% CI)*			
breast milk only	0.72 (0.48, 1.07)	0.76 (0.58, 1.00)	0.61 (0.24, 1.59)	0.66 (0.53, 0.82)
predominantly breast	0.77 (0.52, 1.14)	0.85 (0.65, 1.10)	0.28 (0.08, 0.92)	0.72 (0.59, 0.89)
both equally	0.71 (0.40, 1.25)	1.08 (0.74, 1.56)	0.21 (0.03, 1.46)	0.83 (0.62, 1.12)
predominantly formula	0.94 (0.62, 1.42)	1.25 (0.96, 1.64)	0.91 (0.33, 2.49)	1.08 (0.87, 1.34)
formula only	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)

* OR (95% confidence interval) for overweight (n=1030) v normal weight youth (n=12,203), adjusted for age, gender, Tanner stage. ** 419 had gestational diabetes and 56 had pre-existing diabetes. Further adjustment for maternal BMI, demographics and lifestyle factors, birth weight, gestational age, and offspring lifestyle factors did not materially alter results. **Conclusions:** Breast feeding was inversely associated with childhood obesity regardless of maternal diabetes or weight status. These data provide support for all mothers, including those who are overweight or have diabetes, to breast feed their infants to reduce risk for childhood overweight.

O-070

Bottle Feeding of Sheep Alters Regional Adipose Deposition and Increases Adult Fat Mass David S. Gardner, Helen Budge, Michael E. Symonds; *Centre for Reproduction and Early Life, School of Human Development, University Hospital, Nottingham, UK.*

Background: The early nutritional environment a sheep experiences has been shown to influence its adiposity at birth and as an adult. Poor nutrition during intrauterine and/or early postnatal development encourages greater adipose deposition upon realimentation to a normal diet. Bottle as oppose to breast feeding of human infants has been suggested to render the subsequent adult susceptible to greater body fat gain. No study has examined bottle-feeding of sheep and later health and body composition. The current study therefore examines interactions between gestational and postnatal nutrition, and differing levels of physical activity upon young adult regional adipose deposition in sheep. **Methods**: At day 23 gestation 37 twin-bearing ewes were randomly allocated to receive either a control (C, 7 MJ/day; n=24) or nutrient restricted diet (NR, 50% C intake ~3.5 MJ/day; n=13) from day 30 to 80 gestation. Thereafter all sheep were fed to 100% calculated metabolisable energy requirements to term (12-13 MJ/day near term). Offspring delivered spontaneously and were either ewe reared or bottle-fed (BF, n=8 from control group; 1-1.5 L/d Volac) until weaning (10-12 weeks). From weaning to 1yr of age offspring were either reared indoors with restricted activity and increased food availability to promote fat deposition (Obese controls OC, n=8; Obese nutrient restricted ONR, n=13; Obese bottle-fed OBF, n=8) or pasture grazed with unrestricted activity (Lean controls LC, n=8). At this time all offspring were humanely euthanased (electrocortical stunning with exsanguination) before removal and measurement of all major organs and fat pads. All animal protocols and procedures were locally approved and performed under the UK Animals (Scientific Procedures) Act, 1986. **Results:** Weight of lambs at birth were (LC, 4.32 ± 0.30; OC, 3.32 ± 0.10; ONR, 3.36 ± 0.10; OBF, 4.19 ± 0.40 kg) being 6.8 ± 0.3, 5.9 ± 0.1; 5.7 ± 0.2; 5.6 ± 0.5 % of maternal weight, respectively. After weaning, overfed and physically inactive lambs gained weight rapidly being LC, 42 ± 3; OC, 62 ± 0.2; ONR, 60 ± 2; OBF, 69 ± 2 kg by 6 months and LC, 58 ± 4; OC, 90 ± 2; ONR, 89 ± 1; OBF, 99 ± 4 kg at 12 months of age. At post mortem, by definition obese lambs (OC, ONR & OBF) had more adipose than lean lambs (LC; see Table). However, OBF had significantly more perirenal adipose than other obese groups. When each region was expressed as a % of total measured adipose then OBF had significantly more perirenal and significantly less omental fat than all other nutritional groups (Table).

	Lean	Obese			
	LC	OC	ONR	OBF	*P*
Perirenal fat (g)	553 ± 93^{a}	2692 ± 294^{b}	2783 ± 197^{b}	3661 ± 214^{c}	0.000
Pericardial fat (g)	82 ± 8^{a}	328 ± 29^{b}	232 ± 29^{b}	341 ± 54^{b}	0.000
Omental fat (g)	785 ± 95^{a}	4222 ± 154^{b}	3598 ± 201^{b}	3788 ± 316^{b}	0.000
% perirenal (% total)	38.1 ± 2.3^{a}	36.6 ± 2.8^{a}	41.8 ± 1.6^{ab}	47.4 ± 2.3^{b}	0.01
% pericardial (% total)	6.9 ± 1.4	4.5 ± 0.3	3.7 ± 0.6	4.3 ± 0.6	0.07
% omental (% total)	54.9 ± 2.6^{ab}	58.8 ± 2.7^{b}	54.4 ± 1.5^{ab}	48.2 ± 2.5^{a}	0.03
Subcut fat (11th rib)	4.3 ± 1.0^{a}	21.5 ± 4.4^{b}	22.1 ± 1.5^{b}	29.2 ± 2.5^{b}	0.000

Key: Values within a row not sharing a superscript are significantly different at P<0.05 (1-way ANOVA). **Conclusions:** Sheep that were exposed to an environment in which physical activity is reduced and intake of energy dense food is uninhibited rapidly become overweight when compared to their pasture-grazed counterparts. As young adults they are obese. However, bottle feeding from birth to weaning promotes further gain in mass, particularly of adipose tissue. This extra adipose is not deposited evenly but rather is preferentially laid down around the kidneys at the expense of omental adipose. Thus bottle feeding at the infant stage not only influences overall fat mass but also regional deposition.

O-071

BMI Throughout the Life-course and Blood Pressure in Mid-adult Life Leah Li, Chris Power, Catherine Law; *Centre for Pediatric Epidemiology and Biostatistics, Institute of Child Health, UCL, 30 Guilford Street, London,WC1N 1EH, UK*

Background. There are many studies of BMI and blood pressure, but few examine the contribution of BMI at different life stages. Overweight and obesity in childhood have been increasing in most industrialised countries; hence, it is important to understand the implications of this trend for cardiovascular risk factors in adult life. Studies of BMI at different stages of child and adult life are needed to establish risks for adult blood pressure. Our aim was to investigate the association between BMI during different periods of life, from childhood to adulthood, and blood pressure in a nationwide population cohort followed from birth to 44y. **Methods** We use data from the 1958 British birth cohort, all born in one week in March 1958. Outcomes were systolic and diastolic blood pressure for 4617 men and 4651 women measured at age 44y. Birthweight and body mass index (BMI, weight(kg)/height(m)^2) at ages 7, 11, 16, 23, 33, and 44y were converted to internally derived standard deviation scores. We applied multiple regression models to assess the effect on systolic and diastolic blood pressure of birthweight, BMI, and change in BMI at different stages of childhood and adult life. **Results.** Birthweight and current (44y) BMI were associated with blood pressure, but in opposite directions; a SD decrease in birthweight was associated with an increase in systolic blood pressure for men of 0.65 mmHg (95% CI: 0.13 to 1.17) and, for women, of 1.84 mmHg (95% CI: 1.32 to 2.36); whereas, a SD increase in current (44y) BMI was associated with an increase in systolic blood pressure for men of 3.57 mmHg (95% CI: 3.13 to 4.01) and, for women, of 4.18 mmHg (95% CI: 3.74 to 4.62). These associations strengthened in mutually adjusted models. Associations of BMI at each age were examined separately and effects were seen to increase with age: among men for example, the increase in systolic blood pressure associated with a one SD in BMI at age 7y was 0.55 mmHg (95% CI: 0.04 to 1.06), strengthening to 3.57 mmHg (95% CI: 3.14 to 4) for BMI at age 44y. We next examined changes in blood pressure per year for each time interval, ie. 7-11y, 11-16y, 16-23y, 23-33y, 33-44y. Change in BMI at each life stage was positively associated with blood pressure. For every one SD unit gain in BMI per year during the interval between ages 7 and 11, systolic blood pressure was elevated for males by 3.28 mmHg (95% CI: 0.48 to 6.08) and for females by 4.80 mmHg (95% CI: 2.08 to 7.52). A greater effect was seen in relation to BMI gain for the interval between ages 33 and 44y: a one SD gain in BMI per year during this period was associated with an elevation in systolic blood pressure for men of 37 mmHg (95% CI: 29.3 to 44.7), and for women, of 49.8 mmHg (95% CI: 41.44 to 58.16). The importance of BMI gain was confirmed using tests for interaction between BMI at several ages. A similar pattern of results in relation to birthweight, BMI and gain in BMI at different life stages was found for diastolic blood pressure. In further analysis of onset of overweight, we found that those who were overweight in childhood but not as an adult had similar systolic and diastolic blood pressure on average to those who had never been overweight. **Conclusions.** BMI and gain in BMI at any life stage are strongly associated with blood pressure in adult life. In this nationwide British population, BMI gain in adulthood has particularly important effects on blood pressure, but BMI gain in childhood was also a relevant factor. Individuals with the lowest weight at birth had higher systolic and diastolic blood pressure in mid-adult life than those with heavy weight at birth.

Parallel Session 5B: Immune Function / Respiratory Outcomes

O-072

Lower Birth Weight is Associated with Poorer Lung Function but not Asthma Diagnosis in 25839 British Telecom Employees Anna A. Davies, George Davey Smith, Yoav Ben-Shlomo, Dean A. Sivell, Paul Litchfield; Department of Social Medicine, University of Bristol, Canynge Hall, Whiteladies Road, Bristol, BS8 2PR, UK. St George's, University of London, Cranmer Terrace, London SW17 0RE, UK. BT Group plc, 81 Newgate Street, London, EC1A 7AJ, UK.

Background. Studies investigating the effect of early life events on respiratory outcomes report conflicting results: the lack of large studies in this field limiting conclusions. This study investigates whether birth weight predicts lung function and asthma in a large adult population. **Methods**. Between 1994 and 1996 British Telecom (BT) employees were invited to participate in a voluntary occupational health screening. All exposures were self-reported by questionnaire. Peak expiratory flow rate (PEFR) was measured by trained occupational health nurses using standard procedures. The association between birth weight and outcome variables was assessed using linear or logistic regression analysis adjusted for possible confounding factors.

Characteristic	Men (N=18288)	Women (N=7551)
Age (years)	38.9 (7.4)	35.9 (8.6)
Body mass index (kg/m^2)	25.3 (3.2)	24.0 (4.0)
Peak expiratory flow rate (l/min)	586.9 (70.2)	443.6 (58.0)
Birth weight (kg)	3.4 (0.6)	3.3 (0.6)
Participants of white ethnicity (%)	92.3	83.8
Participants in management roles (%)	37.2	21.8
Participants diagnosed with asthma (%)	7.6	7.9
Participants who smoke (%)	14.9	18.2

Results. 132000 BT employees undertook the health screening of which 45122 (34.2 %) had both questionnaire and clinic data. Birth weight, PEFR and lifestyle data were available for 25839: the large majority of those excluded at this stage was because of

failure to report birth weight. The table reports the general characteristics of the study population by sex. General characteristics of the British Telecom study population (N=25839). Values are mean (SD) or percentage distribution. Mean (SD) PEFR was significantly lower in women compared with men ($p<0.001$) and in asthmatics *vs.* non-asthmatics (521.7 (98.6) vs. 547.0 (92.6); $p<0.001$). A 1kg increase in birth weight was associated with 11.6 l/min increase in PEFR after adjustment for age and sex (95% confidence interval 10.3 to 13.0 l/min; $p<0.001$). The association remained significant but reduced to 4.9 l/min per 1 kg increase in birth weight after additional adjustment for adult height (95% CI 3.6 to 6.3 l/min; $p<0.001$). Further adjustments for BMI, socio-economic position, physical activity, alcohol intake, and smoking status did not alter the findings. Neither the removal of non-white participants (2633 participants, 10.2%) nor premature babies (<1.5kg, 184 participants, 0.7%) from the analysis affected any associations. There was no interaction between birth weight and sex to predict PEFR. In a logistic regression, adjusted for age, sex and BMI, birth weight did not predict asthma diagnosis (Odds Ratio 1.0; 95% CI 0.9 to 1.1). **Conclusions.** In this large study population birth weight was an important predictor of adult lung function as measured by PEFR. Further adjustment for adult confounding factors did not alter the strength of this association. The lack of data on childhood respiratory diseases is one limitation of this study. Further large scale studies are required to test whether such postnatal factors may in part or wholly explain the association observed.

O-073

Infant Weight-for-Length as a Predictor of Wheeze in Early Childhood

Elsie M. Taveras,[1] Sheryl L. Rifas-Shiman,[1] Diane R. Gold,[2] Carlos A. Camargo,[2] Emily Oken,[1] Scott Weiss,[2] and Matthew W. Gillman.[1,3] *[1]Department of Ambulatory Care and Prevention, Harvard Medical School and Harvard Pilgrim Health Care; Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School; and Department of Nutrition, Harvard School of Public Health; all in Boston, MA*

Background: Cross-sectional studies have reported a positive association between measures of fatness and prevalence of asthma and wheeze among children. Few prospective data link early childhood growth with asthma-related symptoms. The objectives of this study were to examine the associations of weight-for-length z-score at age 6 months with incidence of any wheezing, recurrent wheezing, and doctor-diagnosed reactive airway disease by age 3 years. **Methods**: We studied 588 infants and toddlers in Project Viva, an ongoing prospective cohort study of pregnant mothers and their children. The main outcome measures were parents' report of 1) any wheezing (or whistling in the chest) from 6 months to age 3 years, 2) recurrent wheezing during the first 3 years of life, and 2) doctor's diagnosis of reactive airway disease (i.e. asthma, wheeze or reactive airway disease) by age 3. When the infants were 6 months of age, we measured their length and weight and calculated age- and sex-specific weight-for-length z-scores based on US CDC reference data. We used multiple logistic regression to examine associations between 6-month weight-for-length z-scores and our main outcomes by age 3 years. **Results**: At 6 months, infants' mean (SD, range) weight-for-length (WFL) z-score was 0.71 (0.97, -2.96 to 3.24). By age 3 years, 32% of children had any wheezing, 12% of children had recurrent wheezing and 16% had doctor-diagnosed reactive airway disease. After adjustment for several potential confounders, we found that that the higher the 6-month WFL z-score, the greater the odds of having had any wheezing or recurrent wheezing by 3 years of age (Table). WFL z-score at 6 months also appeared to be associated with slightly greater odds of doctor-diagnosed reactive airway disease at age 3 years. Birth weight was not associated with any of the outcomes.

Multivariate Model*	Any Wheezing	Recurrent Wheezing	Doctor-diagnosed Reactive Airway Disease
	Adjusted Odds Ratio (95% Confidence Intervals)		
6 month WFL z-score (1-unit)	1.32 (1.06, 1.66)	1.45 (1.02, 2.07)	1.18 (0.90, 1.54)

* Adjusted for child's birth weight, sex and passive exposure to smoking during the first year of life; mother's age, race/ethnicity, pre-pregnancy BMI, and breastfeeding status at 6 months; household income; number of children under 12 years of age living in the household; and maternal and paternal history of asthma

Conclusion: In this prospective study, infants who were heavier at 6 months of age had a greater risk of wheezing by age 3 years. Our findings suggest that early interventions to moderate excess infant weight gain may help reduce children's risk of asthma-related symptoms.

O-074

Early Life Origins of Spontaneous Hypothyroidism in Adult Women

Eero Kajantie, David I.W. Phillips, Clive Osmond, David J.P. Barker, Tom Forsén, Johan G. Eriksson; National Public Health Institute, 00300 Helsinki, Finland; *MRC Epidemiology Resource Centre, University of Southampton, Southampton SO16 6YD, UK; Developmental Origins of Adult Health and Disease Centre, University of Southampton, Southampton SO16 6YD, UK*

Background. Previous studies have suggested that thyroid autoantibodies occur more frequently in adults who were born with low birthweight. Whether body size at birth and during childhood is related to overt thyroid disease in adulthood has not been studied. **Methods**. We measured free thyroxine (fT4) and thyrotropin (TSH) concentrations in 293 women who were born in Helsinki, Finland (a region of mild to moderate iodine deficiency), between 1934 and 1944, and lived there during childhood. Their measurements at birth and during childhood were recorded. At examination, the subjects were interviewed for thyroid disease, the diagnosis of which was confirmed from medical records. Women with a history of other thyroid disorders were excluded. 18 women (6.1%) had been diagnosed spontaneous hypothyroidism and were taking thyroxine. Age at diagnosis ranged from 43 to 65 years. A further two had undiagnosed latent hypothyroidism (TSH>10 mU/l with normal fT4). Of these 20 women (6.8%) with spontaneous hypothyroidism, 16 (80%) were TPO antibody positive as compared with 59 (22%) of those with normal thyroid function. **Results**. Compared with women with no thyroid disease, the 20 women with spontaneous hypothyroidism were born at a later gestational age (286 vs. 281 days, 95% CI for difference 0.3 to 10.0 days; $p=0.04$), had lower birth weight (3161 vs. 3426 g; 95% CI for difference 72 to 458 g; $p=0.007$ adjusted for gestational age) and shorter length at birth (49.0 vs. 50.3 cm; 95% CI for difference 0.5 to 2.0 cm; $p=0.001$). The odds of developing hypothyroidism increased 4.8-fold per kilogram decrease in birth weight (95% CI 1.5 to 15.3). Hypothyroid subjects had been shorter in early childhood and had had lower BMI during later childhood (Fig.). At examination, they were shorter (160.8 vs. 163.6 cm; $p=0.04$) but had a similar body mass index ($p=0.7$). **Conclusions**. Spontaneous hypothyroidism during adulthood is predicted by small size at birth and during childhood. These novel observations introduce autoimmune thyroiditis among those late-life disorders whose development is initiated during early development.

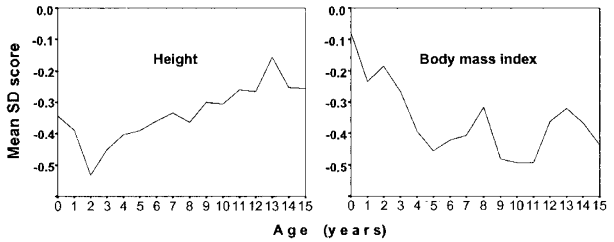
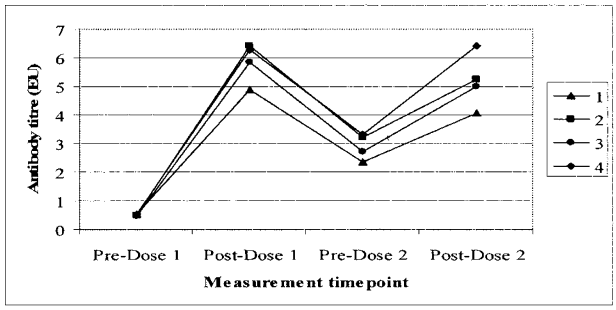


Figure. Relative height and weight (in SD units) during childhood in women who developed spontaneous hypothyroidism in adulthood. The mean height and BMI of the cohort are set at zero. p for height < 0.05 between 1 and 6 years; p for BMI <0.05 at 4 and 5 and between 10 and 15 years.

O-075

Revaccination Does Not Improve Observed Deficit in Antibody Responses in Pakistani Adults Born of a Lower Birth Weight Sophie E. Moore[1], Fehmida Jalil[2], Andrew M. Prentice[1] & Lars Å. Hanson[3]. *1. MRC International Nutrition Group, London School of Hygiene & Tropical Medicine, London, UK. 2. King Edward Medical College, Lahore, Pakistan. 3. Department of Clinical Immunology, Göteborg, Sweden.*

Background: We have previously shown that the antibody response to a polysaccharide typhoid vaccine is impaired in Pakistani adults born of a lower birth weight (Moore *et al. Am J Clin Nutr* 2004 **80**,453-9). Since no correlation with size at birth was observed in response to a protein (rabies) vaccine, we concluded that the generation of antibodies to polysaccharide vaccines, which have greater B-cell involvement, is compromised by fetal growth retardation. In the current study we revaccinated study subjects to test whether this observed deficit is alleviated with a booster dose of the same vaccine. **Methods**: A total of 228 Pakistani adults (mean age 31.1y, males 57%) were given a second single dose of a Vi polysaccharide vaccine for *Salmonella typhi* (Typhim Vi, Aventis Pasteur). Antibody titres were measured in serum samples collected at baseline and then 21 (± 2.8) days following vaccination. **Results**: Pre-vaccination antibody titres were strongly correlated to post-vaccination titres from the previous study ($r=0.832$). Geometric mean (95% confidence interval) antibody titres were 2.86 (2.38-3.46) Elisa Units (EU) before vaccination and 5.10 (4.28-6.09) EU 21 days post-vaccination ($p\leq0.0001$). Response to vaccination was not influenced by current age, gender, body mass index or month of vaccination. Post vaccination antibody titres were positively correlated with birth weight ($F=4.87$, $p=0.0284$). The Figure illustrates the trend in antibody response to both doses of the vaccin by quartiles of the birth weight distribution.



Conclusions: The findings from the current study indicate that adults born with lowest birth weights had a poorer antibody response to vaccination with a polysaccharide antigen vaccine, even following a second booster dose of the vaccine. This finding supports our main research hypothesis that immune development can be impaired in early life, resulting in long-term functional deficits. Specifically, this finding indicates an early-life programming effect on the generation of antibodies during a T-cell

independent immune response. Further studies will explore the specific immunological mechanisms underlying this observation. We acknowledge the Nestlé Foundation and Aventis Pasteur for their support of this study.

Parallel Session 5C: Metabolism in Pregnancy

O-076

The Effect of Maternal Growth Hormone Treatment on Fetal Growth and Adiposity in Rapidly Growing Adolescent Sheep Jacqueline Wallace[1], Masatoshi Matsuzaki[1,2], John Milne[1] and Raymond Aitken[1]; Rowett Research Institute[1], Aberdeen, AB21 9SB, UK and National Agricultural Research Center for Kyushu Okinawa Region[2], Kumamoto 861-1192, Japan

Background: Adolescent girls who continue to growth while pregnant have a high risk of prematurely delivering low birth weight infants. Similarly, when pregnant adolescent sheep are overnourished, maternal tissue synthesis is promoted at the expense of the conceptus, resulting in placental and fetal growth restriction. Maternal growth hormone secretion is attenuated in these ovine pregnancies and we have recently demonstrated that exogenous GH administration throughout the period of placental proliferation stimulates uteroplacental and fetal development at day 81 of gestation (term =145 days). The present study aimed to determine whether these effects persist to term and establish whether GH influences fetal growth and body composition by increasing placental size or by altering maternal metabolism. **Methods**: Adolescent ewes were implanted with singleton embryos from the same sire and a small pool of donor ewes on day 4 post-estrus. Thereafter three groups of adolescent dams were offered a high (H) dietary intake and were either injected twice daily with recombinant GH (bGH, gifted by Monsanto, 0.14mg/kg/day) from day 35 to 65 (H + early GH) or day 95 to 125 (H + late GH) of gestation or remained untreated (H). A fourth moderate intake group acted as optimally nourished controls (C). Pregnancies were either terminated at day 130 of gestation (n=6 per group) or allowed to progress to term (n=8-10/group). **Results**: GH concentrations were elevated (P<0.01) in C compared with H dams throughout pregnancy as reported previously. Exogenous GH administration elevated (P<0.001) maternal GH, insulin, glucose and non-esterified fatty acid concentrations during the defined treatment windows while urea concentrations were decreased (P<0.01). GH treatment reduced maternal adiposity score, the percentage fat in the maternal carcass, internal fat depots and leptin concentrations, predominantly in the late GH group (Table). At day 130 of gestation, placental weight was lower in H versus C dams but independent of GH treatment. In contrast, fetal weight was elevated by late GH treatment and these fetuses had higher relative carcass fat content, perirenal fat mass and liver glycogen stores than all other groups (Table). In pregnancies proceeding to term, the duration of gestation, fetal placental mass and lamb birth weight were reduced in H compared with M dams (P<0.001) but were not significantly affected by bGH treatment.

Table: Selected indices of maternal and fetal adiposity and fetal weight at day 130 of gestation

	Moderate	High	H + early GH	H + late GH
Maternal external adiposity score	2.5 ± 0.04^{c}	3.1 ± 0.06^{a}	3.0 ± 0.06^{ab}	2.9 ± 0.08^{b}
% fat per maternal carcass	25.3 ± 2.64^{c}	41.5 ± 1.57^{a}	36.6 ± 1.13^{ab}	35.8 ± 0.71^{b}
Maternal plasma leptin (ng/ml)	3.1 ± 0.61^{c}	13.3 ± 1.93^{a}	11.2 ± 1.19^{ab}	8.1 ± 1.05^{b}
Fetal weight (g)	4696 ± 211^{a}	3031 ± 411^{b}	2960 ± 245^{b}	3840 ± 292^{ab}
Fetal carcass fat content (g/kg)	27.1 ± 0.96^{b}	30.1 ± 1.13^{a}	29.5 ± 2.05^{a}	42.1 ± 5.17^{a}
Relative perirenal fat mass (g/kg)	4.4 ± 0.14^{b}	5.3 ± 0.43^{b}	5.7 ± 0.24^{b}	7.5 ± 0.70^{a}
Fetal liver glycogen conc.(mg/g)	49.1 ± 4.80^{ab}	42.6 ± 4.84^{a}	47.1 ± 5.51^{a}	64.2 ± 6.79^{b}

Within rows means with differing superscripts differ at P<0.05

Conclusion: Exogenous bGH has major effects on maternal endocrinology, metabolism and body composition when administered during both early and late pregnancy. Treatment during late pregnancy has a modest effect on fetal growth independent of placental size and a profound effect on fetal adiposity which may have implications beyond the fetal period. *Funded by the Scottish Executive Environment and Rural Affairs Department*

O-077

Anthropometry and Glucose/Insulin Concentrations in Indian Children - Relationships to Maternal Gestational Diabetes GV Krishnaveni[1], JC Hill[2], SR Veena[1], SD Leary[2], J Saperia[2], A Saroja[1], SC Karat[1], CHD Fall[2] *[1]Research Centre, Holdsworth Memorial Hospital, Mysore 570021, India. [2]MRC Epidemiology Resource Centre, Southampton, SO16 6YD, UK.*

Background: To test the hypothesis that the environment experienced by fetuses of mothers with gestational diabetes (GDM), and those with higher glucose concentrations even in the normal range, causes increased adiposity, and altered glucose/insulin metabolism in childhood. **Methods:** Children (n=630), whose mothers were tested for glucose tolerance during pregnancy had detailed anthropometry at birth and annually thereafter. Plasma glucose and insulin concentrations were measured in a 2-hour oral glucose tolerance test in children and in fasting blood samples in fathers at 5 years. **Results:** At birth, offspring of diabetic mothers (ODM, N=41) were larger in all body measurements than controls (babies of non-diabetic parents). At 1 year, these differences had diminished and were not statistically significant. At 5 years, female ODM had larger subscapular (7.4 v 6.3 mm) and triceps skinfold thickness (9.3 v 8.1 mm, p<0.05 for both) than control girls. They also had higher 30- (210 pmol/l v 153 pmol/l in controls) and 120-minute insulin concentrations (122 pmol/l v 90 pmol/l in controls, p<0.05). IGT was more common in the ODM (11% v 3%, p=0.01) than controls. Newborns of diabetic fathers were *lighter* than controls, but showed no difference in anthropometry at 5 years. They had *lower* 120-minute insulin concentrations (63 pmol/l v 83 pmol/l in controls). In control children, maternal insulin was positively associated with skinfolds (triceps 7.8 in lowest v 8.7 mm in highest quartile of maternal insulin, P=0.02; subscapular 6.1 v 6.6 mm, P=0.08) and 30-minute insulin concentrations (152 v 224 pmol/l, P<0.001), and paternal insulin was related to skinfolds (triceps 7.6 v 8.1 mm, P=0.08; subscapular 6.1 v 6.2 mm, P=0.02*) independent of maternal or paternal skinfolds and socio-economic status. **Conclusion**: Maternal GDM increases the risk of adiposity and insulin concentrations in female offspring at 5 years. The absence of similar associations in offspring of diabetic fathers suggests a programming effect of the diabetic intra-uterine environment. With increasing levels of obesity and IGT among Indian mothers, these effects may be contributing to the rise of type 2 diabetes in India. Our continuing follow-up aims to determine the long-term effects of higher maternal glucose concentrations in the absence of GDM.

O-078

Effects of a Low Protein Diet on Pancreatic Development of NOD Mice Astrid Chamson-Reig, Edith Arany and David Hill Lawson Health Research Institute, Departments of Physiology and Medicine, University of Western Ontario, London, ON, Canada

Introduction. The non-obese diabetic (NOD) mouse is an animal model that has been used for the study of Type I diabetes as it spontaneously develops the disease following the autoimmune destruction of ß-cells in the pancreatic islets. By the age of 8-12 weeks the pancreas shows lymphocytic infiltration around the islets (insulitis). Progression from insulitis to diabetes is typically associated with T helper 1 (Th1) pancreatic inflammation. Human and rodent studies have shown that early exposure to different sources of proteins is an environmental risk factor for the development of diabetes. Using a model of maternal protein restriction, we have shown a decrease in the incidence of insulitis and a delay on the onset of diabetes in pups exposed to a low protein (LP) diet during fetal and neonatal development. We also demonstrated that the serum levels of interferon gamma (INF-γ, a Th1 cytokine) were reduced by the low protein diet both at a prediabetic stage (8 weeks) and at the onset of diabetes. **Objectives**. Our aim was to characterize the main changes occurring on the pancreas of NOD mice after the exposure to a protein restricted diet in early life. Based on the previous results we focus this study on the morphology and function of the pancreas. **Methods**. NOD breeding pairs were obtained from the Robarts Research Institute (University of Western Ontario). Mice were mated and, after confirming pregnancy, were divided in two groups. The first group was fed a 20% protein diet (C, n = 7 litters), and the second one received an isocaloric 8% protein diet (LP, n = 8 litters); after weaning (21 days-old) all pups were fed with the 20% protein diet. The pregnant mice and the pups were weighed regularly. At 8 weeks of age, normal onset of insulitis for this colony, some pups were sacrificed. The remaining pups were monitored for the onset of diabetes by checking glycosuria, and sacrificed after confirming diabetes by hyperglycemia, or at 45 weeks of age. Blood samples were collected for RIA measurement of insulin. Pancreata were dissected and fixed in 10% formalin for immunohistochemistry (IHC) or stored in RNAlater for RNA and protein extractions. Parameters as mean islet area, and percentages of endocrine, alpha or beta cells were determined by IHC followed by image analysis. **Results**. At 8 weeks the incidence of insulitis was reduced on the LP group compared to C group (females C: 10.03 ± 3.64 % vs females LP: 3.58 ± 1.75 %, p<0.05). The percentage of pancreas occupied by endocrine cells was reduced on the LP females (C: 0.664 ± 0.085 % vs LP: 0.328 ± 0.066 %, p<0.05). The changes in the percentage of endocrine cells correlates with a reduction in alpha and beta cells as well. The serum levels of insulin were decreased in the LP females (C: 0.16 ± 0.02 ng/ml vs LP: 0.11 ± 0.02 ng/ml) and males. **Conclusion.** We conclude that the exposure to a protein restriction during fetal life could lead to morphological (reduction in β-cell mass) and functional (reduction in serum insulin levels) alterations in the pancreatic development. The low protein diet may reduce the susceptibility of the pancreas or beta cells to the immune system by a decrease in its function.

O-079

Reduced Beta Cell Function in Offspring of Mothers with Young Onset Type 2 Diabetes R Singh[1], E Pearson[1], PJ Avery[2], MI McCarthy[3], JC Levy[3], GA Hitman[4], M Sampson[5], M Walker[2], AT Hattersley[1]; [1]Peninsula Medical School, Exeter, UK, [2]University of Newcastle upon Tyne, Newcastle, UK, [3]University of Oxford, Oxford, UK, [4]University of London, London, UK, [5]Norfolk & Norwich University Hospital, Norwich, UK

Background: Animal models indicate that even exposure to mild maternal hyperglycemia in-utero is detrimental to offspring beta cell function, but there is limited evidence of this in humans. Risk of diabetes is greatly increased in offspring of Caucasian mothers diagnosed with type 2 diabetes (T2D) <50yrs.
Aim: We hypothesized that Caucasian offspring born to mothers who developed young-onset T2D would be exposed to mild hyperglycemia in-utero (either through gestational diabetes, prediabetes or type 2 diabetes) and this would have a detrimental impact on their ß-cell function in adulthood. Offspring born to affected fathers would be suitable controls as they would inherit similar genetic susceptibility but without additional programming. **Method**: We analysed early insulin response (EIR), insulin resistance using HOMA%S, and HbA1c from 578 non-diabetic adult offspring born to parents with T2D (mean age 55.8 yrs) split according to which parent was affected (327 mothers) and parental age of diagnosis: <50 yrs (n=117) or ≥50 yrs. **Results**: EIR

was lower in offspring with young-onset maternal T2D compared with young-onset paternal T2D (log EIR 4.32, 95%CI: 4.14 – 4.51 vs 4.63, 95%CI: 4.43 – 4.83, p=0.02). HbA1c was increased in the young-onset maternal diabetes offspring (4.89%, 95%CI: 4.79 – 4.99 vs 4.68%, 95%CI: 4.57 – 4.79, p=0.002). Insulin sensitivity was similar in the two groups. **Conclusion**: We conclude that offspring of mothers with young-onset T2D have ß-cell dysfunction as a result of exposure to mild maternal hyperglycemia consistent with programming adding to an inherited genetic susceptibility to diabetes.

Parallel Session 5D: Genetic / Epigenetic Influences

O-080

Strain Differences in the Impact of Maternal Dietary Restriction on Fetal Growth, Pregnancy and Postnatal Development in Mice Brian Knight[1,2], Craig Pennell[1,3], Stephen Lye[1,2]. [1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; [2]Obs/Gyn and Physiology, University of Toronto, Toronto, ON, Canada; [3]School of Women's and Infants' Health, The University of Western Australia, Perth, Australia.

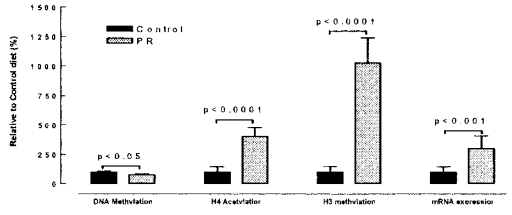
Objective: The association between sub-optimal intrauterine environment and development of adult disease (DOHaD) is variable suggesting that genotype may contribute to eventual outcome. Chromosome substitution stains of mice allow the genetic dissection of complex phenotypic traits; however, to date, they have not been utilized to evaluate the genetic contribution to DOHaD. The objective of this study was to characterize maternal, fetal and adult offspring responses to dietary restriction (DR) during pregnancy in two genetically different strains of mice. **Methods:** Fourteen A/J and 14 C57BL/6J (B6) pregnant mice underwent 30% DR from 6.5 days (d) until 18.5d of gestation: a further 14 mice in each strain served as controls on an *ad libitum* diet. Half of the animals were dissected on 18.5d to assess fetal weights, organ weights and maternal endocrine status. The remaining mice were allowed to deliver spontaneously and their offspring (n=78) were randomly allocated to either an *ad libitum* control diet or a high fat/sucrose (HF) diet. Growth, body composition and glucose tolerance were assessed at 3 months of age. **Results:** DR resulted in reduced fetal body weight in both strains (-17%; p=0.01). Fetal kidney weight was reduced in both strains (p=0.001); however, the magnitude of the reduction was 3-fold greater in B6 vs. A/J (-19% vs. -7%; p=0.04). DR-B6 when compared to DR-A/J had: significantly overgrown placentas, increased fetal brain-liver ratio and decreased fetal-placental weight ratio (all p=0.05). Maternal cortisol at day 18.5 was increased 2-fold in the DR-B6 (p=0.02) whilst no change was seen in DR-A/J mice. DR resulted in a reduction in placental expression of 11B-HSD2 in both strains (-36%; p=0.02); however an increase in 11B-HSD1 protein expression was only seen in DR-B6 mice (+21%; p=0.011). DR was associated with pre-term delivery in both strains with DR-B6 delivering earlier than DR-A/J (18d vs. 19d; p=0.01). Postnatal growth patterns were different between the two strains of mice exposed to maternal DR during pregnancy. At 3 months of age DR-A/J mice on the postnatal *ad libitum* diet were still smaller than controls (-11%, p=0.01) whereas the DR-B6 mice had catch up growth to controls (+7% above controls). The addition of a postnatal HF diet did not alter the growth trajectories in control-B6, DR-B6 or control-A/J; however, a postnatal HF diet induced a significant increase in body weight in the DR-A/J (+34%, p<0.01) when compared to DR-A/J on a control diet. PIX*lmus* densitometry scans of body composition at 3 months demonstrated a significant increase in %fat in both strains (control-A/J 25%, DR-A/J 29%, p<0.05; control-B6 23%, DR-B6 29%, p<0.05) on postnatal *ad libitum* diet. The addition of a postnatal HF diet further increased body fat in the DR-A/J to 35% (P<0.01) but no change was seen in the DR-B6. Maternal DR did not alter glucose tolerance at 3 months in either strain and there was no effect of postnatal dietary modification. **Conclusion:** Maternal, fetal and adult offspring responses to DR during pregnancy in A/J and B6 mice are different and the observed strain variations may offer a unique opportunity to investigate gene:environment interactions associated with DOHaD.

O-081

Maternal Dietary Protein Restriction During Pregnancy Induces Altered Epigenetic Regulation of the Glucocorticoid Receptor in the Liver of the Offspring after Weaning Karen A. Lillycrop[1], Alan A. Jackson[2], Mark A. Hanson[3] and Graham C. Burdge[2]; [1]Development and Cell Biology, [2]Institute of Human Nutrition and [3]DoHaD Centre, University of Southampton, Southampton, UK

Background. Feeding a protein-restricted (PR) diet during pregnancy in the rat induces a phenotype characterised by hypertension, insulin resistance and dyslipidaemia in the offspring. The PR diet during pregnancy increased expression of the glucocorticoid receptor (GR) in the liver of the offspring by reducing the methylation status of the exon 1_{10} promoter [1]. Such epigenetic changes to the regulation of gene expression provide a causal mechanism linking unbalanced prenatal nutrition to persistent alterations to the metabolic phenotype of the offspring. Altered GR expression confers greater sensitivity to glucocorticoids. In addition, since the GR possesses demethylase activity [2], increased GR expression may induce persistent changes to the regulation of target genes [1]. Long-term changes to the regulation of gene expression also involves covalent modifications to the structure of histones, and such alterations to histone structure are required for the transcription of unmethylated gene promoters. We therefore determined the effect of feeding a PR diet during pregnancy in the rat on the acetylation of histone H4 and methylation of histone H3 (on K4) in the GR exon 1_{10} promoter in the liver of the offspring after weaning. **Methods**. Rats were fed either a control (18% casein) or PR (9% casein) diet from conception to delivery, then standard chow (AIN-76A) during lactation [3]. Litters were reduced to 8 at birth and offspring were weaned onto chow at postnatal (pn) day 28. Offspring were killed at pn day 34, and livers frozen in liquid nitrogen. GR promoter methylation was determined (n=6/group) by methylation-sensitive real-time PCR and GR mRNA expression was measured by semi-quantitative RT PCR [1]. Histone modification at the GR promoter was assessed using chromatin immunoprecipitation assays [4] with antibodies raised specifically against acetylated histone H4 and di-methylated histone H3 (K4). **Results**. Feeding the PR diet during pregnancy decreased GR promoter methylation (23%), increased histone H4 acetylation (301%), increased histone H3 methylation (1025%) and increased mRNA expression (200%) (Fig. 1) **Discussion**. Maternal PR diet during pregnancy leads to an increase in offspring liver histone H4 acetylation and H3 K4 methylation at the GR promoter. Both histone acetylation and methylation of K4 on histone H3 are modifications associated with transcriptional activity, consistent with increased GR in the offspring liver. These observations suggest that alterations to chromatin structure contribute to altered gene expression and lead to altered offspring phenotype.

Figure 1



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O-082

Postnatal Loss of Igf2 Imprinting in Rat Offspring Born After Maternal Undernutrition During Pregnancy Marie Dziadek, Susan Ravelich, Greg Smith, Selina Patel, Galina Konycheva, Stefan Krechowec, Mark Vickers[1], Bernhard H. Breier[1], Mik Black[2]; *School of Biological Sciences, University of Auckland, Liggins Institute[1] and Department of Statistics[2], University of Auckland, Auckland, New Zealand.*

Epigenetic programming is an important mechanism that regulates the growth and development of the mammalian fetus and placenta, and underlies the process of genomic imprinting, which results in parental allele-specific gene silencing. To determine if fetal programming of adult disease is associated with epigenetic alterations, we have investigated whether the establishment of allele-specific expression of imprinted genes during embryonic and fetal development is influenced by maternal undernutrition during pregnancy. Female rats were maintained throughout pregnancy from day 1 on either an *ad libitum* chow diet (AD) or on 30% of the AD food intake (UN, undernourished). Newborn offspring from UN mothers were cross-fostered onto AD females. Wistar females were mated with Lewis males; strains in which we identified single nucleotide polymorphism (SNP) differences within Igf2 and H19 transcripts. Placental and fetal tissues were dissected at E20 of gestation and postnatal tissues from offspring at 21 days after birth (P21). RNA was extracted and cDNA prepared from several tissues from at least 6 individual animals in each group. PCR-based SNP allelic discrimination assays (Applied Biosystems) were developed to detect the expression of maternal (Wistar) and paternal (Lewis) alleles of Igf2 and H19 in individual tissues. Expression levels of imprinted genes were also quantified in RNA pooled from tissues of either AD or UN offspring using custom oligo microarrays. Maternal undernutrition reduced fetal (1.99 ± 0.18 vs 2.45 ± 0.16 g for AD) and placental (0.39 ± 0.05 vs 0.49 ± 0.07 g for AD) weights at E20 ($p < 0.01$ for each) and bodyweight at P21 (39.5 ± 5.0 vs 52.9 ± 2.0 g for AD, $p<0.01$). Paternal allele-specific expression of Igf2 was maintained in all tissues (including liver, muscle, heart, kidney, lung, placenta) from both AD and UN conceptuses at E20, except for the choroid plexus, which showed the expected biallelic expression. Likewise, maternal allele-specific expression of H19 was observed in all tissues at E20 except the choroid plexus. At P21, biallelic expression of Igf2, but not H19, was found in kidney and lung tissues from 4 of 6 UN offspring but not in tissues from AD offspring, indicating loss of Igf2 imprinting in the former. Biallelic Igf2 expression was not, however, associated with increased Igf2 transcript levels. Other tissues (muscle, liver, heart) retained paternal allele-specific Igf2 expression and maternal allele-specific H19 expression in both AD and UN offspring at P21. This study provides the first *in vivo* evidence for reactivation of the silenced allele of an imprinted gene by changes in maternal nutrition during pregnancy. Three conclusions can be drawn from these outcomes: 1. Loss of Igf2 imprinting is not evident until after birth, indicating that maternal undernutrition most likely predisposes offspring to epigenetic instability that manifests in the postnatal period. 2. Susceptibility to loss of imprinting (LOI) is tissue-specific, and is seen in tissues in which LOI associated cancers have been described in humans (Wilms' tumour, lung cancer). 3. LOI and biallelic expression of Igf2 is not inevitably associated with increased expression of Igf2 in the postnatal period. We suggest that

LOI of Igf2 represents a general epigenetic lability in offspring programmed by maternal undernutrition and does not necessarily translate at this age into increased Igf2 expression that would have phenotypic consequences.

O-083

Intrauterine Growth Retardation Affects Postnatal Histone H3 Methylation and Subsequent Histone – Individual Gene Interactions in a Gender and Tissue Specific Manner Robert A. McKnight, Xingrao Ke, Qi Fu, Xing Yu, Robert H. Lane; Division of Neonatology, University of Utah School of Medicine, Salt Lake City, UT 84158 USA

Background: Uteroplacental insufficiency (UPI) induces intrauterine growth retardation (IUGR). Using a rat model of UPI and IUGR, our group has previously demonstrated that IUGR causes persistent alterations in brain and liver gene expression, which correlate with altered DNA methylation and histone H3 acetylation. These alterations occur in a gene, tissue, and gender specific manner, which suggests specificity consistent with the histone code hypothesis. Maintenance of the histone code also involves histone methylation. **Hypothesis**: We therefore hypothesized that uteroplacental insufficiency and subsequent IUGR alters perinatal and postnatal histone methylation in brain and liver. We further hypothesized that these alterations can be used to identify, in IUGR rats, genes with altered expression due to the subsequent changes in histone-DNA interactions. **Methods**: UPI and IUGR were induced by bilateral uterine artery ligation at day 19 of gestation in pregnant Sprague – Dawley rats. Pups from sham operated dams were used as controls (Con). Day 0(D0) pups were harvested by caesarian section at term. Postnatal animals were allowed to deliver spontaneously and were harvested at either day of life 21(D21) or 120 (D120). Histone modifications were quantified using Western blotting, with total histone as an internal control. Chromatin immunoprecipitation (ChIP) and differential display PCR (DD-PCR) was performed as previously described using antibody to differentially methylated histones and random primers respectively. Unique DD-PCR products were cloned and sequenced. mRNA levels of specific genes were quantified using rea-time RT-PCR. Gene expression changes within the brain were localized by immunohistochemistry (IHC). (n = 6 litters for brain;n = 8 litters for liver) **Results**: mRNA and protein levels are expressed as percent of Con animals. In the **brain**, IUGR significantly decreased H3 Lysine 9 (H3/K9) dimethylation in both D21 male (53 ±1% ***) and female rats (68.8 ± 4.1% **). Similarly, H3/K9 trimethylation was decreased in D21 IUGR male brains, whereas H3/K9 trimethylation was significantly increased in the D21 IUGR female brains. Brain ChIP identified **HoxB9** and the **catalytic subunit of telomerase (Tert)** as genes affected by IUGR. Western blotting demonstrated that IUGR significantly altered the protein levels of both genes. IHC localized these changes primarily to the hippocampus. In the **liver**, IUGR significantly increased H3/K9 dimethylation in D0 pups (180 ± 12.5%**) and D21 male pups (127 ± 7%*). H3/K4 dimethylation (159 ± 9.5%**) and H3/K27 di-trimethylation (181 ± 9.3%**) were also increased in IUGR male livers, whereas H3/K4 dimethylation was significantly decreased in IUGR female livers (77 ± 4.4%*). ChIP identified **eukaryotic elongation factor-2 kinase (eEF-2K)** as a gene affected by IUGR. Hepatic eEF-2K mRNA levels were significantly decreased in IUGR male animals at D0, D21, and D120. (*p < 0.05, ** p < 0.01, ***p < 0.001) **Conclusion**: UPI and IUGR affect histone H3 methylation in a tissue and gender specific manners. and we were able to use the affected H3 methylation sites to identify genes through ChIP whose postnatal expression was affected by IUGR. The two genes identified, TERT and eEF-2K, are particularly intriguing considering the role TERT plays in protecting neurons against DNA damage induced death, and eEF-2K plays in growth and insulin signaling. We speculate that changes in histone acetylation and methylation form a part of the histone code that contributes to the phenotype of the IUGR rat.

Parallel Session 6A: Prematurity

O-084

Central Adiposity and Low Lean Mass Develop at Young Adult Age in Former Extremely Low Birth Weight (ELBW) Compared to Normal Birth Weight (NBW) Infants *Stephanie A Atkinson, Susan Steele, Barbara Stoskoff and Saroj Saigal, Department of Paediatrics, McMaster University, Hamilton, Ontario, Canada L8N 3Z5*

Background: Programming of body composition may have its origins in fetal and neonatal life. Premature ELBW infants who develop abnormal distribution of body fat and lean mass and low bone mass may be at risk for insulin resistance, cardiovascular disease and osteoporosis. The objective was to compare bone mineral content (BMC), body mass index (BMI), total body fat (TBF), fat-free mass (FFM) and abdominal visceral adipose tissue (VAT) in ELBW and NBW peers at young adulthood (YA). **Design/Methods:** Longitudinal regional cohort study. Participants: Population-based cohort of ELBW survivors of 501-1000 g BW (1977-82), and a sociodemographically matched NBW reference group. Bone, lean and fat mass and abdominal region estimate of visceral adipose tissue (VAT) of the whole body were measured by dual energy x-ray absorptiometry (Hologic QDR1000W and Discovery) and Z-scores for whole body bone mineral content (BMC) were calculated using database from www.bmc.tmc.edc/bodycomlab/ . BMI, fat mass index (FMI) and fat free mass index (FFMI) were calculated by dividing mass by height^2.
Results: At YA 125/166 (75%) ELBW survivors and 119/145 (82%) NBW were assessed at a mean±SD age of 23.1±1.4 and 23.5±1.2 y. Prevalence of BMC z-score < -1.5 (15.2 vs. 6.7%, p<0.04) and < -2.5 (3.2 vs 0%, p<0.05) was greater in ELBW.

Table: Data (mean±SD) are summarized (a > b, p<0.03 by t-test).

	ELBW Male N = 41	NBW Male N = 40	ELBW Female N = 55	NBW Female N= 52
BMC Z-scores	-0.96±0.91[a]	-0.36± 0.31[b]	-0.26± 0.85[a]	0.31± 1.06[b]
BMI, kg/m^2	24.2±4.3	23.4±4.3	23.4±4.2[a]	23.9±4.1[b]
FFMI, kg/m^2	17.7±0.3	18.5±0.5	15.0±0.2	16.0±0.2
% TBF	20.3±6.6[a]	16.6±5.2[b]	30.6±7.1	29.0±6.4
Total VAT in region (g)	1643±1109[a]	1167±735[b]	1969±1295	2035±1538
VAT region (%)	25.6±10.3[a]	18.6±8.6[b]	25.6±10.2	27.4±11.3

Conclusions: Bone mass was low in about 20% of the ELBW at YA. While BMI was similar between groups and genders, % TBF and VAT were greater in the male ELBW compared to NBW group at YA and FFMI was lower in female ELBW. Excess adiposity in the abdominal region and subnormal lean mass in survivors of ELBW at YA may signal a risk for metabolic derangements of dyslipidemia and dysglycemia, and these should be investigated in this population.

O-085

Children Born Preterm Have Lower Functional Skin Capillary Density and Higher Blood Pressure Anna-Karin Edstedt Bonamy, MD; Helena Martin MD, PhD; Mikael Norman, MD, PhD. *Department of Woman and Child Health, Karolinska Institute, Stockholm, SWEDEN*

Background: Adults with essential hypertension have lower skin capillary density and impaired endothelial function, even before hypertension develops. Children and young adults born preterm have higher blood pressure (BP) than controls born at term. This study aims at investigating the relationship between blood pressure, capillary density and endothelial function after preterm birth. **Methods**: 60 school children aged 7-12 years were studied: 19 born very preterm (=30 weeks of gestation) and small for gestational age (preterm SGA), 20 born very preterm and appropriate for gestational age (preterm AGA) and 21 term controls with normal birth weight. Using intra vital videomicroscopy, the dermal capillary density in fingers of the left hand was measured before and after venous occlusion. Endothelial function was assessed using a laser Doppler technique to determine skin perfusion before and after transdermal delivery of acetylcholine (ACh), an endothelium-dependent vasodilator. Brachial blood pressure (BP) was also recorded. **Results**: Preterm children had a lower functional capillary density (i.e. before venous occlusion), 89.9 vs. 96.6 capillaries/mm2 (p=0.042), both before and after adjustment for age and gender. After venous occlusion (measuring structural differences) this difference diminished and was no longer statistically significant, (91.4 vs. 96.8 capillaries /mm2, p=0.12). There was no difference in capillary density between preterm SGA and preterm AGA. Systolic BP was significantly higher in children born preterm and in boys, but no correlation to capillary density was found. Maximum vasodilatory response to ACh did not differ between groups and did not correlate to capillary density. **Conclusion**: Very preterm birth is associated with lower functional skin capillary density and higher blood pressure at school-age. In contrast to fetal growth restriction, there is no link between preterm birth and endothelial dysfunction in childhood.

O-086

Maternal Body Composition Prior to Conception and Age: Relationships with the Duration of Gestation JF Johnstone[1], RM Lewis[5], S Crozier[4], H Inskip[4], M Hanson[5], JRG Challis[123], KM Godfrey[4,5] and the Southampton Women's Survey Study Group. Departments of Physiology[1], Obstetrics and Gynecology[2] and Medicine[3], University of Toronto, Ontario; MRC Epidemiology Resource Centre[4] and Centre for Developmental Origins of Health and Disease[5], University of Southampton, Southampton, UK.

Objective: In sheep, nutritional restriction to produce a 15% reduction in maternal weight before conception is associated with increased preterm delivery and a shorter duration of gestation. In human pregnancy, anthropometric measurements can be used to derive estimates of maternal fat mass and arm muscle area, and these aspects of body composition provide indicators of maternal nutritional status and metabolic capacity. Previously, we found lower activity of 11b HSD-2 in term placentas from thin women with a smaller mid-upper arm circumference before conception; placental 11b HSD-2 activity was also lower in older women. This suggests that the fetuses in these pregnancies may have been exposed to inappropriate levels of maternal cortisol, which is a mediator of gestation length. In this study, we examined the hypothesis that the duration of gestation would be shorter in women who tended to be thin and in those who were older at the time of conception. **Methods:** Within the population-based Southampton Women's Survey (SWS), our analyses were performed on a sample of 392 women whose estimated date of conception was set from menstrual data confirmed by an early ultrasound scan and who had a spontaneous onset of labour and delivered after 259 days or more gestation (37 weeks). In the SWS, the mother's body composition before pregnancy has been measured using detailed anthropometry. We used linear regression to examine the relationships of maternal body composition and age with the duration of gestation. **Results:** Within these term pregnancies, lower maternal body mass index, mid-upper arm circumference and arm muscle area before conception were all associated with shorter duration of gestation at delivery (r=0.11, p=0.024; r=0.10, p=0.05; and r=0.12, p=0.015, respectively). A lower subscapular/triceps skinfold thickness ratio and older maternal age were also associated with shorter duration of gestation (r=0.11, p=0.035 and r=-0.12, p=0.017, respectively). Maternal height and sum of skinfold thickness measurements before conception were not related to the duration of gestation. **Conclusions:** In this study of

term pregnancies, we found that thinner women with a lower mid-upper arm circumference and arm muscle area tended to have a shorter duration of gestation. The mother's adiposity, estimated from the sum of skinfold thicknesses was not, however, related to the duration of gestation, suggesting that it is lower lean mass and a reduced metabolic capacity that influences gestation length. An association between lower subscapular/triceps skinfold thickness ratio and shorter duration of gestation could reflect an effect of body fat distribution. Our findings of shorter gestation length in thinner and older women are in keeping with our previous observation of lower 11b HSD-2 activity in term placentas from thinner and older women. We conclude that in women who deliver at term aspects of the mother's metabolic capacity prior to conception could influence the duration of gestation through mechanisms that include alteration in placental metabolism and fetal cortisol exposure.

O-087

Mild Premature Birth Alters Airway Structure and Function in Later Life K Snibson[1], L McMurtrie[2], R Bischof[1], T Yawno[2], GS Maritzz[3], ML Cock[2], S Hooper[2], R Harding[2]. *[1]Centre for Animal Biotechnology, School of Veterinary Science, University of Melbourne, VIC, Australia; [2]Department of Physiology, Monash University, VIC, Australia; [3]Department of Medical Biosciences, University of the Western Cape, Bellville, South Africa.*

Background: Preterm birth affects up to 10% of all births and has been associated with evidence of airflow limitation and respiratory illness in later life, but mechanisms are unknown. Even mild preterm birth, not requiring ventilatory support, is reported to adversely affect lung function. Hence preterm birth alone, in the absence of iatrogenic factors, could permanently alter lung development such that later lung function and respiratory health are affected. **Aims:** In order to determine the effects of preterm birth per se, we have measured resting pulmonary airflow resistance (R_L) and responsiveness to carbachol challenge (CCh) in mature sheep that were born prematurely in the absence of neonatal respiratory support. **Methods:** Three cohorts of preterm lambs (P) were delivered at 133 days of gestation, the earliest age at which lambs could survive without requiring ventilatory support. Controls (C) were born at term (term~147d). The 3 groups were studied up to (a) term equivalent age (TEA group), (b) 6 weeks after TEA (6wks post-TEA), or (c) to~1 year of age (adult). Lung function was studied in sedated, ventilated animals and lung tissue collected post-mortem. In adult sheep, preterm (n=7) and control (n=8) groups we assessed airway responsiveness from cumulative concentration-response curves to increasing concentrations of inhaled carbachol (CCh). **Results:** At birth, preterm lambs were ~30% lighter than controls but caught up in body weight by 6 weeks after birth; in adults, body weights were not different (P, 41.1±4.1 vs C, 45.3±3.9 kg). Pulmonary resistance was 62% higher in P lambs than controls at TEA, and remained higher at 6 weeks post-TEA. Alveoli were more numerous ($p=0.05$) and smaller ($p=0.05$) in preterm lambs compared to controls at both ages. Alveolar septa were 33% thicker and the alveolar blood-gas barrier was 26% thicker in P lambs than in controls at TEA, and remained thicker at 6 weeks post-TEA. In P lambs, the airway epithelium was thicker at TEA and 6 weeks post-TEA. At 6 weeks post-TEA, dry lung weight and lung protein content were ~50% greater in preterm lambs than in controls ($p<0.05$), whereas lung DNA, elastin and collagen contents were similar in the 2 groups. At ~1 year of age, arterial blood gas tensions at rest did not differ between preterm and control groups. As a group, the adult preterm sheep displayed a trend towards increased baseline R_L (P, 2.6±0.8 vs C, 1.8±0.8 $cmH_2O/sec/l$; $p=0.1$) but no change in airway responsiveness to CCh (ie. 175% increase above baseline R_L) compared to controls (P, 60.6±21.0 vs C, 63.7±8.2 breath units of CCh). When a subset of 3 lighter preterm sheep (31.9±1.0 kg) was compared to controls they showed a significant increase in baseline R_L (3.2±0.5 $cmH_2O/sec/l$; n=3, $p<0.05$) as well as greater airway responsiveness to CCh (14.0±4.9 breath units of CCh, $p<.001$). **Conclusions:** Mild preterm birth, in the absence of ventilatory support, impacts upon lung development; in particular, the airway epithelium and alveolar septa are thicker. The increased airway resistance and responsiveness to CCh in the mature sheep born prematurely that experienced the lowest postnatal growth rates suggests that mild preterm birth per se, followed by restricted postnatal growth, may increase susceptibility to airway hyperresponsiveness.

Parallel Session 6B: Populations in Transitions

O-088

The Effects of Birth Size, Childhood Growth and Adult Anthropometry on Glucose Tolerance, Insulin Resistance and Insulin Secretion in Young South Indian Adults Raghupathy P[1], Antonisamy B[2], Saperia J[3], Leary S[3], Priya G[2], FS Geethanjali[4], Barker DJP[3], Fall CHD[3] *Departments of Child Health[1], Biostatistics[2], Clinical Biochemistry[4], Christian Medical College, Vellore, 632 004, India; MRC Environmental Epidemiology Resource CentreUnit[3], University of Southampton, Southampton General Hospital, Southampton, Hampshire SO16 6YD, UK*

The prevalence of type 2 diabetes is increasing worldwide. By 2025, nearly 57 million people are predicted to be affected by diabetes in India. Rapid changes of urbanization, greater availability of food, reduced physical activity and increase in obesity are thought to be causal. Besides, India with its high incidence of low birth weight may be facing the same risks as Western countries where fetal, infantile and childhood growth parameters have been documented to influence the risk of developing impaired glucose tolerance (IGT) and type 2 diabetes in adult life. Recent studies show that incidence of diabetes is increasing even in rural areas and that IGT occurs as frequently as in urban populations, suggesting a high future burden of disease. The focus of this paper is to study young adults from a rural and urban cohort and to examine the relationships of IGT and diabetes, and insulin resistance and secretion, to parental size, size at birth and in infancy, and growth and BMI gain during childhood. We studied anthropometry, glucose tolerance and insulin profiles in 2,218 young men and women (mean age 28 years; median BMI 20.0 kg/m^2) who were representative of a large population-based birth cohort of 10,691 individuals born during 1969-1973 in Vellore town and nearby rural areas. Their growth was measured at birth and during infancy, childhood and adolescence, as part of a prospective research study of pregnancy outcome. Family history, socio-economic status, physical activity and tobacco and alcohol use were recorded. The subjects had oral glucose tolerance tests (WHO protocol), including measurements of plasma insulin concentrations, from which estimates of insulin resistance and secretion were derived. Multiple linear and logistic regression analyses were done adjusting for age, sex, body mass index and other adult variables. Mean Z scores of BMI at birth (ponderal index), infancy, childhood, adolescence and adulthood in subjects with normoglycaemia and DM/IGT were compared. A high prevalence of type 2 diabetes – 2.8% (men - 2.9%; women - 2.6%) and IGT – 16.3% (14.8%; 18.0% respectively) was observed with urban women showing IGT as high as 22.5%. Higher insulin resistance and lower insulin increment were noted in rural subjects. Current urban residence, positive family history of diabetes, higher BMI and waist/hip ratio, more household possessions, higher education level, higher alcohol intake, and less physical activity were associated with an increased risk of IGT and diabetes or higher insulin resistance. After adjustment for these factors, lower birthweight was associated with a higher prevalence of IGT and diabetes combined (p 0.02) and higher 120-minute glucose concentrations on oral glucose tolerance test (p 0.003). High rates of disease occurred in people who were born small and were light or thin during infancy but who had accelerated weight or BMI gain during childhood. The highest rates of IGT/DM were in subjects who were in the lowest third of BMI in childhood and the highest third as adults (40%) as compared to the highest third of BMI in childhood and lowest adult BMI (8.5%) and highest BMI in childhood and adulthood (27.9%). The figures were similar in adolescence and adulthood. Abnormal glucose tolerance is associated with thinness in childhood followed by accelerated BMI gain through childhood and adolescence. Diabetes is associated with intra-uterine growth restriction. Prevention of accelerated BMI gain at least from childhood onwards would seem to be a robust public health objective.

O-089

The Next Generation: Surveillance of Offspring of Mothers with Youth-Onset Type 2 Diabetes Heather J. Dean, Elizabeth A.C. Sellers, Michael Mendelson, Shayne P. Taback, Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada.

Background: Genetic, intrauterine and postnatal environmental factors contribute to the risk of youth-onset type 2 diabetes (T2D). The odds ratio is 14.4 for maternal pre-gestational T2D in affected youth followed in the regional pediatric diabetes program in Manitoba, Canada. A unique polymorphism in the hepatic nuclear factor 1-alpha gene, HNF-1α G319S, was found in 30% of these Oji-Cree youth with early-onset type 2 diabetes (Sellers et al. Diabetes Care 2002; 25:2202). The relative contributions of this genetic polymorphism, the intrauterine diabetic environment and postnatal environmental factors as well as the natural history of glucose intolerance and T2D in this high risk population are unknown. **Objective**: In 2004 we established a cohort of offspring of First Nation women diagnosed with T2D before age 19 years to assess the feasibility of long-term surveillance and to determine the presence of genetic and modifiable risk factors for T2D in their offspring. **Methods**: Additional eligibility criteria for the mothers included current residence in Manitoba and previous longitudinal follow-up in the regional pediatric diabetes programme. Baseline assessments of the offspring included demographic, anthropometric and environmental risk factors for obesity and T2D. We tested for HNF-1α G319S and fasting blood glucose (FBG) in offspring age ≥3 years. **Results**: Of the 26 mothers, 22 (85%) agreed to enroll their children in long-term surveillance; 3 did not have custody of their children and we could not contact the guardians and 1 mother refused participation. The 38 offspring (18 males) ranged in age 0.1-14.8 years. Of the 17 children aged 2-6 years, 7 (41%) were overweight (BMI 85-94th %ile) and 9 (53%) were obese (BMI ≥95th %ile). Modifiable risk factors in this age group were the consumption of ≥2 cans/day of sugar drinks in 42%, watching ≥2 hours of television per day in 47% and participation in <1 hour/day of physical activity in 14%. Of the 9 children aged 7-15 years, 6 were overweight or obese and 3 had developed T2D at age 9, 11 and 12 years with BMI Z-scores of 1.1, 2.1 and 2.8 respectively. We found no new cases of T2D or impaired fasting glucose. Of the 18 offspring aged ≥3 years, 17 have 1 (n=14) or 2 (n=3) copies of HNF-1α G319S. The 3 offspring with T2D all had 1 (n=1) or 2 (n=2) copies of HNF-1α G319S. **Conclusion**: Long-term surveillance of this high risk cohort is feasible. Obesity is established in early childhood in this high-risk cohort. Lifestyle interventions should be targeted during the prenatal and infancy periods. Novel approaches for the prevention of T2D may need to be considered for carriers of the unique HNF-1α G319S polymorphism.

O-090

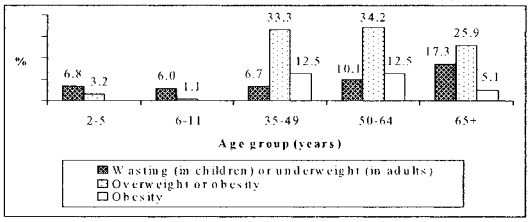
The Challenges of a Prospective Study of an Indigenous Birth Cohort: Aboriginal Birth Cohort 1987-2001 Susan Sayers, Dorothy Mackerras, Gurmeet Singh; *Menzies School of Health Research, Institute of Advanced Studies, Charles Darwin University Darwin, Northern Territory, Australia*

Introduction: There is limited information about the developmental origins of adult health and disease (DOHaD) from indigenous populations. The literature is dominated by western populations, where poor maternal and fetal nutrition rates are low. However non-western cohorts particularity indigenous ones, ideally suited to test the DOHaD hypothesis, present unique challenges relating to their indigeneity which include geographical, physical, cultural and ethical issues. **Aim:** To recruit and follow An Australian Indigenous birth cohort. **Subjects:** 686 maternal child pairs were recruited at birth with a maternal malnutrition rate of 15% (BMI < 18.5 kg/m2 measured within 4 days postpartum) and fetal growth retardation of 25% (birth weight for gestational age < 10^{th} percentile). **Setting:** The cohort participants were all born at the Royal Darwin Hospital but at follow up 11 years later, 70% were living in rural and remote locations in three states of the sparsely populated area of north Australia. **Challenges:** Participants were traced and examined in their local communities in a vast area with poor infra structure. 39% of participants lived in communities without year round road access, due to monsoon conditions and a further 20% lived on islands. Aircraft were used, but limitations of weight, daylight flying hours, weather and costs were all constraints to this form of travel. Cultural influences occurred throughout the study. At the time of delivery, rural mothers were reluctant to stay in the air conditioned multi-storied hospital building and had to be found in the hospital grounds and surrounding areas. Few Aboriginal mothers knew their last menstrual period (6.5%) and only 8.5% had had an early dating fetal ultrasound so a post natal clinical estimation of gestational age was necessary. By the age of 4 years 30% of children had a different name to that recorded on the birth register and 20% had a different address. Multiple identifiers were essential. In an area of 30 different dialects, 75% of families did not speak English in the home. Local interpreters and community advocates both paid and voluntary were used but skin relationships associated with "poison cousins" prevented some community members from talking to others. Flexible research plans were important to allow for the impact of a death and community participation in ceremonies. No mothers refused participation at recruitment raising ethical concerns about lack of confidence or misunderstanding about refusal. At follow-up, 22% of children had non-parent carers, with no formal guardianship authority being given to other carers. Local inquiries usually resulted in instant and unanimous agreement on who could answer for a child. **Results:** At 11 years of age, vital status was determined for 95% of the original cohort and 86% of living children were examined. These outcomes suggest strategies used for tracing participants and interacting with Indigenous communities were successful and that Indigenous community members were supportive of the study.

O-091

Study of Nutritional Transition in the Urban Setting of Ouagadougou (Burkina Faso) Hermann Z. Ouédraogo, Jean Garry, Laeticia Nikièma, Pierre Meyer, Benoit Varenne, Maud Harang, Ali Niakara, Yves Martin-Prevel, Gerard Salem, Florence Fournet. Institut de Recherche en Sciences de la Santé, 03 BP 7192 Ouagadougou, Burkina Faso, Institut de Recherche pour le Développement, 01 BP 181 Ouagadougou, Burkina Faso, Université de Ouagadougou, 03 BP 7023 Ouagadougou, Burkina Faso.

Background: The rapid urbanization of West African cities leads to major environmental and socio-demographic changes. The health consequences of these changes are not yet fully described. The process of nutritional transition results in the presence of both underweight and overweight at a given time. This study aimed at analyzing underweight and overweight in the population of Ouagadougou. **Methods**: We performed anthropometrics measurements on 1298 preschool children (aged 2-5 years), 2063 school-aged children (6-11 years) and 2045 adults (age >35 years) in 1570 households (HH), through a 2 round cross sectional survey (April-June and September-October 2004). We selected these HH from 2 stratums characterized as rich or poor districts. We computed body mass index (BMI) of children and adults and weight-for-height z-score (WHZ) of children. Percents of underweight (BMI<18.5) and overweight (BMI>25) in adults, and percents of wasting (WHZ<-2) and overweight (using the Cole's international cut-off points according to age) in children have been the main expressed results. We established logistic regression models to identify predictors of wasting in children and overweight in adults. **Results**: The figure below presents proportions of wasting, underweight, overweight and obesity.



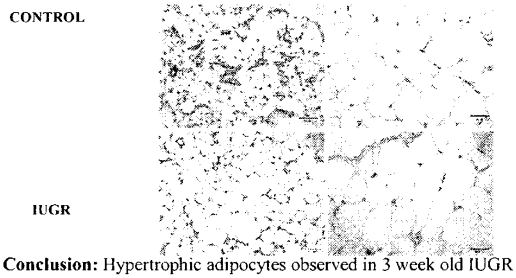
The main predictors of wasting in children were dry season (OR=1.59 [1.15-2.20]), diarrhea (OR=2.37 [1.36-4.11]) and acute-low-respiratory-infection (OR=1.59 [1.05-2.41]). Those of overweight in adults were rich districts (OR=1.70 [1.32-1.98]), lack of physical activity (OR=2.68 [1.94-3.72]), female gender (OR=2.76 [2.08-3.65]), and age of 35-64 years (OR=1.78 [1.11-1.85]). Underweight and overweight have coexisted within 6.6% of HH. The proportion of HH with an overweight children was 5.5% and 4.7% among HH with and without an overweight adult respectively (p=0.546). Among HH with an underweight member, 31.5% also had an overweight member. **Conclusions**: Overweight is the appendage of adults, particularly female adults, in rich districts. Undernutrition in children remains an important problem and is associated to infectious diseases. Nutritional interventions so far focusing on children's undernutrition should also simultaneously address adults overweight in the urban setting of Ouagadougou. They should take the coexistence of overweight within underweight HH into account.

Parallel Session 6C: Obesity and Body Composition

O-092

Altered Cellular Adipocyte in Programmed Obese Offspring of Food-Restricted Rat Dams Mina Desai[1], Nathash Kallichanda[2], Monica Ferrini[3], Dave Gayle[1] and Michael G. Ross[1]; [1]*Department of Obstetrics and Gynecology, [2]Department of Pathology, [3]Department of Urology, LABioMed at Harbor-UCLA Medical Center, and David Geffen School of Medicine at UCLA, Torrance, CA 90502, USA*

Background: Epidemiologic studies have demonstrated that gestational undernutrition leads to increased risk for offspring development of adult metabolic syndrome, characterized by obesity, diabetes, and cardiovascular disease. The mechanism for this effect is not well understood. We have developed a rat model of 50% maternal food restriction in pregnancy, resulting in intrauterine growth restricted (IUGR) pups with decreased plasma leptin levels at 1 day of age. When provided normal nutrition via *ad libitum* nursing, IUGR pups demonstrate rapid catch-up growth. By 3 weeks of age these offspring demonstrate increased body weight and plasma leptin levels, though normal body fat as compared to controls. By 9 months of age, these offspring have markedly increased body weight, body fat and plasma triglyceride levels and, demonstrate clinically evident leptin and insulin resistance. The finding that leptin is elevated prior to the increase in body fat suggests, that abnormalities of adipocytes may be the underlying mechanism involved in the later development of obesity. We sought to determine adipocytes cell size in fat pads of IUGR and Control offspring. **Methods:** Pregnant Sprague Dawley rats and offspring were studied. Control dams (n=6) received *ad libitum* food, whereas study dams (n=6) were 50% food-restricted from pregnancy day 10 to 21 to produce IUGR newborns. At birth, litter size was culled to 4 males and 4 females. All pups were nursed by dams fed *ad libitum* and were weaned at 3 weeks to *ad libitum* feed. At ages 3 weeks and 9 months, epididymal fat pads were dissected, fixed in 4% phosphate-buffered formalin, embedded in paraffin, sectioned and stained using hematoxylin-eosin stain. Image PRO software (version 5.1) was used to determine the area of adipocytes (5 pictures per section, and 2 sections per animal). Values shown are mean±SE from male offspring. **Results:** At 3 weeks of age, adipocyte cell volume of IUGR pups was 45% greater than controls (693±67 vs 383±49 μm^2, $p<0.01$) with proportionately reduced cell number. At 9 months of age IUGR adipocyte cell volume was 25% greater than control (IUGR=5275±165 vs 3985±107 μm^2, $p<0.01$) as shown in Figure below.



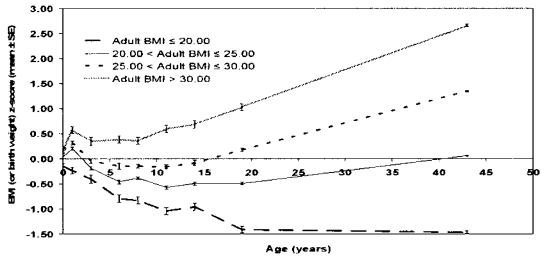
Conclusion: Hypertrophic adipocytes observed in 3 week old IUGR pups following rapid newborn weight gain persist through adulthood. We speculate that enhanced storage of triglycerides occurring in thrifty phenotype offspring provides a competitive advantage in preparation for a nutrient-deprived environment. Under conditions of nutrient availability, the thrifty phenotype inadvertently results in adult obesity.

O-093

Life Course BMI Growth Pattern in Danish Overweight and Obese Adults Lene Schack-Nielsen[1], Erik L. Mortensen[2], Kim F. Michaelsen[1], June M. Reinisch[3], Thorkild I.A. Sørensen[3]; [1]Department of Human Nutrition and Centre for Advanced Food Studies, The Royal Veterinary and Agricultural University DK-1958 Frederiksberg C, [2]Department of Health Psychology, Institute of Public Health, University of Copenhagen, DK-2200 Copenhagen N, [3]Danish Epidemiology Science Center, Institute of Preventive Medicine, Copenhagen University Hospital, DK-1357 Copenhagen K, Denmark

Background: Several studies suggest that fast BMI increase is a risk factor for childhood obesity, but studies reaching into adulthood are sparse. **Methods**: The Copenhagen Perinatal Cohort consists of 9125 individuals born at the Copenhagen University Hospital in 1959-1961. Weight and height were available from follow-up examinations at birth, 1, 3, and 6 y, from school health records at 8, 11, and 14 y, from draft board examination for men (mean age±SD 19.2±1.3 y) and from a mailed

questionnaire at the age of 42-44 y (n = 4306). BMI measurements and birth weight (BW) were transformed into z-scores also adjusting BW for gestational age (The British Growth Reference 1990). Growth pattern in underweight, normal weight, overweight and obese adults were compared by ANOVA and LSD tests as appropriate. **Results**: The BMI growth pattern is shown in the figure. Group differences were significant at all ages (P<0.001) and LSD tests showed that from the age of 1 y all groups were significantly different (P=0.023 at 1 y, P=0.019 at 3 y, P=0.003 at 6 y and thereafter P<0.001 at all ages). Dividing 1y BMI into quartiles confirmed that these differences persisted; adult BMI ±SD were 24.1±4.2, 24.6±4.0, 25.3±4.5, 26.0±4.4 kg/m^2 (P<0.001) in the first, second, third, and fourth 1 y BMI quartile, respectively. The pattern was similar, but weaker, if dividing into BW z-score quartiles; 24.7±4.3, 24.8±4.3, 25.0±4.5, 25.4±4.5 kg/m^2 (P=0.004), respectively. The variation in growth was larger during infancy than from 1 to 3 y (64 vs. 50 %, P<0.001, who had a ? z-score >0.67 or < -0.67 during infancy and from 1 to 3 y, respectively).



Conclusion: Our data suggest that development of overweight or obesity is related to size differences present at birth that are further developed and stabilised during infancy and childhood. The majority of children change percentile during infancy and this could be a sensitive period with potential long-term consequences. Therefore increased knowledge of early determinants of weight gain may be important for preventing lifelong weight and obesity.

O-094

Size at Birth, Lean and Fat Body Mass and Fat Distribution at 9-10 Years of Age

Imogen S Rogers, Andrew R Ness, Colin Steer, Jon Tobias, *ALSPAC, Department of Community-based Medicine, University of Bristol, MRC Childhood Nutrition Research Centre, Institute of Child Health, London, Clinical Science at South Bristol, Bristol UK*

Background: Birthweight has been positively associated with BMI and the risk of overweight in later life. This seems to be in conflict with the inverse associations between birthweight and the risk of many chronic obesity-related diseases, and it has been suggested that the positive associations between birthweight and subsequent BMI may be driven by increases in lean body mass (LBM) rather than fat mass. However, there is little information on how birth weight is associated with subsequent lean and fat body mass, and on how other measures of size at birth such as birth length and ponderal index (PI) relate to later body habitus. **Objective**: The aim of this study was to investigate the association between weight, PI and length at birth, and LBM, fat mass and fat distribution at age 9-10y. **Design**: This study used data from subjects (n = 3006 boys and 3080 girls) participating in the Avon Longitudinal Study of Parents and Children. Weight and length at birth were measured or taken from hospital records. Total LBM, fat body mass and trunk fat were measured using Dual-Energy X Ray Absorptiometry. **Results:** On adjusting for current height, birthweight was positively associated with LBM at age 9-10y in both sexes, with LBM rising by around 600g per kg increase in birthweight ($p < 0.001$), and also positively associated with fat mass and the fat:lean ratio in girls only. PI at birth was positively associated with LBM, fat mass and the fat:lean ratio in both sexes The fat:lean ratio increased by around 2% to 3% per kgm^{-3} increase in PI at birth ($p < 0.001$). Increased length at birth was associated with a reduced fat:lean ratio at 9-10 years. There was no association between size at birth and central adiposity in the sample as a whole – although trunk fat was strongly positively associated with PI at birth, these associations disappeared on adjustment for total fat mass. Associations between size at birth and later body habitus were largely unchanged on adjustment for measures of social position and maternal factors. **Conclusions:** Higher PI at birth is associated with increases in both fat mass and LBM at age 9-10 years, but also with an increase in the ratio of fat:lean mass. PI at birth is a better predictor of subsequent adiposity than birthweight.

Table 1. Percentage change in the fat:lean ratio at 9-10y per unit change in birthweight, PI at birth and birth length

	Boys			Girls		
	B (95% CI)	p	R^2	B (95% CI)	p	R^2
Birthweight (kg)	0.7 (-3.4, 4.9)	0.747	0.119	3.8 (0, 7.8)	0.050	0.089
PI (kgm^{-3})	2.3 (1.2, 3.3)	<0.001	0.171	2.9 (2.1, 3.8)	<0.001	0.114
Birth length (per 10cm)	-7.4 (-9.3, -0.1)	0.048	0.142	-9.6 (-9.9, -8.6)	<0.001	0.089

Regression coefficients are adjusted for duration of gestation, current age, height and height^2

O-095

Birth Weight and Birth Length are Predictors of Adult Body Size and Composition in Swedish Women

Petra H. Lahmann[1,2], Lauren Lissner[3], Göran Berglund[2]. [1]Dept. of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbrücke, Nuthetal, Germany; [2]Department of Clinical Medicine, Malmö University Hospital, Lund University, Lund, Sweden; [3]Department of Primary Health Care, University of Göteborg, Sweden

Background. There is good evidence that birth weight (BW), a marker of intrauterine growth, is directly associated with relative weight (BMI) in later life, although the evidence seems to be less strong for middle-aged subjects. Findings from studies that examined body composition data, i.e. more direct measures of adiposity, are less consistent. BW tends to be positively associated with lean body mass (LBM) and inversely associated with fat mass. Other studies indicate an inverse association between BW and fat distribution. We used data from a Swedish cohort to examine the associations between perinatal factors and adult anthropometric and body composition measures, and to identify predictors of attained body size. **Methods.** This study used data from an ongoing case-control study nested within the Malmö Diet and Cancer (MDC) cohort study and was based on archived birth record data from 708 women born in the city of Malmö, Sweden, between 1924-1950. 131 of these women subsequently developed breast cancer, but all were free of breast cancer at the time of their baseline examination (1991-1996). Birth characteristics, including gestational age, and maternal information were abstracted from the available birth records. Adult characteristics and body measures were obtained from the database of the baseline examination including data on body composition assessed by means of bioelectrical impedance analysis (BIA). We used analysis of variance (GLM), correlational and regression analyses to investigate the associations between birth measures and adult body size and composition with or without adjustment for other early life and adult factors. BW was modelled both as a categorical (<3000g, 3000-3499g, 3500-3999g, >=4000g) and continuous variable (by 100g). **Results.** On average women were 55 years old (range 45-71) at study entry when body measures were assessed. The mean (SD) BW and birth length (BL) was 3449g (±524) and 51.4 cm (±2.4), respectively. Most women (98%) were born after 37 completed weeks of gestation; 17% of the subjects had birth weights under 3000g. BW and BL, adjusted for gestational age and attained age, were significantly ($p<0.001$) and positively correlated with adult weight (BW r=0.13; BL r=0.10) and height (BW r=0.26; BL r=0.35), but not with BMI. BW and BL were also significantly and positively correlated with lean body mass (LBM), whereas not with fat mass or %body fat. None of the tested birth measures were correlated with any of the fat distribution measures. Women who weighed over 4000g at birth had a significantly higher adult weight (71.8 kg) compared to women who had a BW less than <3000g (66.2 kg), independent of gestational age, attained age, age at menarche and own occupation (trend across BW categories p=0.003). Similarly, women in the highest BW category were on average 4 cm taller than those in the lowest BW category (162.9 cm) (trend $p<0.001$). Adult LBM increased with each higher BW category (trend $p<0.001$), also after adjustment for height (trend p=0.015). Regression analyses indicated that weight was predicted by BW, age at menarche, and own occupation. Height was predicted by BW, gestational age, attained age, and own occupation. When BL was included in this model, BW lost its significance. BW was a significant predictor for LBM in the multivariate regression model and remained so with inclusion of height, or both height and weight, while the variance explained by BW became smaller. **Conclusions.** In this cohort of Swedish middle-aged women, BW and BL were positively associated with adult weight, height and LBM, but not with BMI, fat mass, or fat distribution.

Parallel Session 6D: Oxidation / Endothelium / Vasculature

O-096

The Effect of Maternal Protein Restriction in Rats on Cardiac Fibrosis and Myocardial Capillarisation in Adult Offspring

Kyungjoon Lim, Monika A. Zimanyi, M. Jane Black; *Department of Anatomy & Cell Biology, Monash University, Clayton, Victoria, 3800, Australia*

Background: Epidemiological studies link intrauterine growth restriction with the induction of ischemic heart disease later in life. The aim of this study was to examine the effect of intrauterine growth restriction, due to maternal protein restriction during pregnancy and lactation, on the level of cardiac fibrosis, myocardial capillarisation and media to lumen ratio of intramyocardial arterioles in the heart of offspring in adulthood. **Methods**: Female Wistar Kyoto rats were fed either a normal protein diet (NPD; 20% casein), or a low protein diet (LPD; 9% casein), during pregnancy and for two weeks after birth. Female offspring were grown to adulthood. At 24 weeks of age a comprehensive evaluation of the levels of interstitial/reparative and perivascular fibrosis was undertaken in both the right and left ventricles using image analysis. In addition, using stereological techniques the length and surface area densities, total length and surface area and average diffusion radius of myocardial capillaries was determined. **Results**: Body weights at 2 weeks and 24 weeks of age were significantly reduced (31% and 8%, respectively) in the LPD offspring. The heart weights and ventricular wall volumes were not different between groups. Importantly, there was a significant 15% increase in fibrosis in the interstitium of the left ventricle in the LPD offspring but no difference in the right ventricle. There were no differences in levels of perivascular fibrosis. Myocardial capillary surface area and length density and total surface area and length were not different between groups. **Conclusion**: Maternal protein restriction during pregnancy and lactation does not affect the capillarisation in the heart at adulthood but importantly leads to increased levels of interstitial fibrosis in the left ventricle. Since cardiac fibrosis is associated with impaired cardiac contractility and arrhythmia, our results suggest that this may be a likely contributor to the increased cardiac disease in adulthood in subjects that were intrauterine growth restricted.

O-097

Quantification of the Fetoplacental Vasculature of an Intrauterine Growth-Restricted Mouse Model using Micro Computed Tomography Monique Y. Rennie [ab], Kathie J. Whiteley [c], Carole S. Watson [c], S. Lee Adamson [cd] and John G. Sled [ab]; [a]*Mouse Imaging Centre, The Hospital for Sick Children,* [b]*Department of Medical Biophysics, University of Toronto,* [c]*Samuel Lunenfeld Research Institute, Mount Sinai Hospital,* [d]*Departments of Physiology and Obstetrics & Gynecology, Univ. of Toronto, Toronto, Canada*

Background: The purpose of this study was to apply micro computed tomography (microCT) to analyze the structural differences in the vascular trees of transgenic (Tg) intrauterine growth restricted and wild type (WT) placentas. The need for novel methods to investigate the normal and abnormal vasculature in the mouse placenta circulation has been emphasized through the recent availability of mutant strains of mice with placental insufficiencies. We have recently demonstrated the first use of microCT as a valid technique for imaging and analyzing the developing vascular tree in murine fetoplacental circulation. Here we aimed to apply the method to observe placental insufficiencies associated with fetal growth restriction. We have demonstrated that fetal growth restriction occurs in Tg mice which have increased fetal circulating insulin-like growth factor binding protein-1 (IGFBP-1). Insulin-like growth factors (IGFs) are critical to fetal and placental growth and their actions are inhibited by specific IGF binding proteins (IGFBPs). Previous work on these mice has shown altered placental hemodynamics similar to those seen in IUGR human fetuses, indicating impaired placental function. **Methods:** The arterial or venous fetoplacental vasculature was perfused with Microfil, a radio opaque silicone rubber contrast agent, at E15.5. The specimens were scanned in a microCT scanner to produce data sets with a voxel size of 13μm. Reconstruction produced three dimensional images and enabled generation of geometric models of the lumen surface, excluding capillaries. Vessel diameter measurements were made using 3D visualization software in 9 Tg and 8 WT specimens.

Results:

	Arterial Diameter (mm)		Venous Diameter (mm)	
	Tg	WT	Tg	WT
Umbilical	0.46 ± 0.03	0.49 ± 0.06	0.39 ± 0.04*	0.46 ± 0.05
Generation 1	0.38 ± 0.04	0.37 ± 0.04	0.36 ± 0.06*	0.42 ± 0.04
Generation 2	0.29 ± 0.04	0.27 ± 0.03	0.27 ± 0.03**	0.34 ± 0.04
Generation 3	0.22 ± 0.03	0.21 ± 0.03	0.18 ± 0.04**	0.25 ± 0.03

*P<0.01, **P<0.001. Values are shown as mean ± standard deviation.

At E15.5, Tg umbilical vein diameter was 20% smaller than WT controls. Venous diameters were also decreased over the 3 subsequent generations of branching. No differences were observed between Tg and WT arterial diameters. **Conclusions:** MicroCT was able to detect quantitative differences between Tg and WT placental vessels. Elevated IGFBP-1 in Tg mice causes reduced venous diameter in the fetoplacental vessels, which could reduce the flow of nutrient rich blood to the fetus. Thus, fetal growth restriction due to elevated circulating IGFBP-1 may be partly due to impaired placental function in the form of reduced venous diameter.

O-098

Muscarinic Receptors Are Key Determinants in Developmental Origins of Pulmonary Endothelial Dysfunction Tolsa J-F, Muehlethaler V, Marino M, Diaceri G, Moessinger A, Peyter A-C.; Neonatal Research Laboratory, Division of Neonatology, Department of Pediatrics, University Hospital CHUV, Lausanne, Switzerland

Background: Perinatal adverse events are associated with the occurrence of chronic diseases in adulthood, such as cardiovascular diseases or diabetes. A common feature of these diseases is that they are all characterized by some degree of endothelial dysfunction. Chronic pulmonary vascular diseases and abnormal pulmonary vasoreactivity in adulthood may also be associated with a hypoxic insult occurring around birth. The muscarinic receptors to acetylcholine (ACh), an endothelium-dependent relaxing agent, and the endothelial nitric oxide synthase (eNOS) are determinants of the effects of ACh on vascular tone. In pulmonary arteries (PAs), the muscarinic receptor isoform M1 (M1AChR) is implicated in the vasconstrictive effect of ACh, whereas the vasodilator effect of ACh is mediated by the muscarinic receptor isoform M3 (M3AChR). **Objectives**: To determine long-lasting effects of perinatal hypoxia on the lung circulation, and to investigate mechanisms implicated in abnormal pulmonary vasoreactivity in adults born in hypoxia, with particular attention to endothelium-dependent and endothelium-independent relaxation. **Methods**: Mice were exposed to hypoxia during the last 5 days of gestation and the first 5 days of life, and then bred in normoxia until adulthood. Adult mice were anesthetized, and closed chest measurements of Right Ventricular Pressure (RVP) were obtained. Isolated pulmonary artery reactivity, biochemical and molecular assays related to the nitric oxide/cyclic guanosine monophosphate (NO/cGMP) pathway were tested at adulthood. **Results**: RVP measured in normoxic conditions (21% O_2) was significantly higher in adult mice born in hypoxia (26.11 ± 0.19 mmHg) as compared to controls (24.95 ± 0.08 mmHg). Exposure to acute hypoxia (12% O_2) during hemodynamic measurements resulted in a significant increase of RVP in both groups, which was even significantly higher in animals born in hypoxia (29.58 ± 0.19 mmHg) than in controls (26.58 ± 0.08 mmHg). The maximal relaxation (Emax) induced by ACh in PAs of mice born in hypoxia was significantly decreased by about 25% as compared to controls (57.61 ± 4.74 % and 75.52 ± 4.67 %, respectively). In contrast, endothelium-independent relaxation induced by NO was similar in the perinatal hypoxia and control groups. ACh-induced relaxation was completely inhibited in the presence of 4-DAMP, a selective antagonist of the M3AChR or in the presence of nitro-L-arginine, an inhibitor of NOS. Pirenzepine, a preferential inhibitor of M1AChR, abolished the effects of perinatal hypoxia on ACh-induced relaxation, and M1AChR mRNA expression was increased in lungs and PAs of adult mice born in hypoxia as compared to controls (56.45±14.61% and 23.49±4.73%, respectively). **Summary**: Transient perinatal hypoxic insult results in altered pulmonary circulation and permanent modifications of the NO/cGMP signalling pathway in the pulmonary vasculature, which could influence adult pulmonary vasoreactivity. The major alterations are found in the endothelium, with a predominant role for the M1AChR. This suggests that muscarinic receptors could be key determinents of "perinatal imprinting". Funded by the Swiss National Foundation (grant 32-67046.01), the Emma Muschamp Foundation, and the Leenaards Foundation.

O-099

Peri-conceptional Dietary Restriction Impairs Femoral Conduit Vasodilatation in Adult Sheep Offspring T.H. Snelling[1] C. Torrens[1], S.K. Ohri[2]. D.E. Noakes[3] L. Poston[4], M.A. Hanson[1] & L.R. Green[1]. [1]Centre for Developmental Origins of Health & Disease, University of Southampton, SO16 5YA; [2]Wessex Cardiothoracic Unit, Southampton General Hospital, SO16 6YD; [3]Department of Veterinary Reproduction, Royal Veterinary College, UK, AL9 7TA; [4]Division of Reproductive Health, Endocrinology & Development, GKT Hospitals, London, SE1 7EH.

Background: Unbalanced maternal nutrition throughout pregnancy and in early gestation effects vascular reactivity in fetal (Nishina *et al.*, 2003; *J. Physiol* **553**, 637-647) and post-natal life (Brawley *et al.*, 2003, *Pediatric Research* **54**, 83-90). The effects of nutritional restriction before, or in very early, gestation on adult offspring vascular function are not known. In this study we investigated the effects of pre- and peri-conceptional nutrient restriction on responses to isolated femoral conduit artery reactivity in adult sheep. **Methods:** Welsh Mountain ewes (UK Animals (Scientific Procedures) Act, 1986) were fed 100% nutrient requirements (control, C, n=8), 50% total nutrient requirements for 30 days prior to conception (PRE, n=12) or 50% total nutrient requirement for 15 days before and 15 days after conception (PERI, n=12) and 100% thereafter. At 3.5 years of age female offspring were euthanized with sodium pentabarbitone (0.8 ml/kg i.v.) and second order femoral artery branches (1 mm) were dissected and mounted in a wire myograph. Reactivity was assessed with cumulative concentration-response curves to phenylephrine, the thromboxane mimetic (U46619), endothelin, angiotensin II, acetylcholine (ACh), bradykinin (BK), isoprenaline and sodium nitroprusside (SNP). Data are expressed as mean ± S.E. and differences were analysed by two-way analysis of variance with Bonferroni *post-hoc* correction for multiple comparisons. Significance was accepted if $p<0.05$. **Results:** There was no significant difference in vasoconstriction between the three groups. Vasodilatation to isoprenaline was significantly impaired in the PERI group compared to the control ($P<0.05$). There were no significant differences in vasodilatation to ACh, BK or SNP. **Conclusions:** This data demonstrates that peri-conceptional nutritional restriction markedly impairs b-adrenergic-mediated vasodilatation in conduit femoral arteries. The absence of this effect in the pre-conceptional restricted group supports the idea that the timing of the restriction is important in determining adult cardiovascular function. *This work was supported by the British Heart Foundation & Hope*

NEW INVESTIGATOR AWARD FINALISTS' ABSTRACTS

N-001

Cardiovascular Stress Responses in Late Life Are Predicted by Gestational Age at Birth Kimmo Feldt, Katri Räikkönen, Johan Eriksson, Sture Andersson, Clive Osmond, David JP Barker, David IW Phillips, Eero Kajantie; Department of Psychology, 00014 University of Helsinki, Finland, National Public Health Institute, 00300 Helsinki, Finland, MRC Epidemiology Resource Centre, University of Southampton, Southampton SO16 6YD, UK, Developmental Origins of Adult Health and Disease Centre, University of Southampton, Southampton SO16 6YD, UK.

Background: The association between small body size at birth and elevated blood pressure in adulthood, although well established, is surprisingly modest given the strong relationships of perinatal variables with frank hypertension and its complications. Recent studies suggest that a substantial role is played by heightened stress *reactivity*, and that these responses may as well be programmed antenatally. **Objective**: To evaluate whether gestational age and birth weight, as markers of fetal environment, predict blood pressure responses during psychosocial stress in late life. **Design:** Clinical birth cohort study. **Methods:** 73 men and 80 women born after 36 weeks' gestation in Helsinki, Finland during 1934-44, underwent a brief standardized psychosocial stress test (Trier Social Stress Test). Blood pressure was monitored on a beat-to-beat basis via non-invasive finger photoplethysmography (Finometer, FMS, The Netherlands). Reactivity scores were determined as the increment from baseline to the mean level during task. Data abstracted from birth records included weight, length and head circumference and gestational age based on last menstrual period. **Results:** Mean systolic/diastolic blood pressure responses were 40/21 (± 17/8) mmHg in men and 41/23 (± 19/9) mmHg in women. The most robust early determinant of blood pressure response was gestational age at birth, which however showed opposite relationships in men and women (p for interaction for systolic=0.001 and diastolic=0.001 responses). After controlling for potential confounders, gestational age was inversely associated with blood pressure responses in women (B=-3.04, 95% CI -1.64 to -4.44, p=0.033 for systolic and B=-1.23, 95% CI -1.89 to -0.58, p=0.062 for diastolic responses). In men, the association was the opposite: (B=4.69, 95% CI 3.31 to 6.07, p=0.001 for systolic and B=2.37, 95% CI 1.73 to 3.01, p=0.001 for diastolic responses). **Conclusions:** Normal variation in gestational age at birth predicts cardiovascular stress responsiveness 60 years later, with opposite effects in men and women. Since the hypothalamic-pituitary-adrenal axis is known to be involved in the regulation of autonomic nervous system function and the timing of parturition, and shows well-established sex differences, we speculate a role of early programming of this axis in explaining this finding.

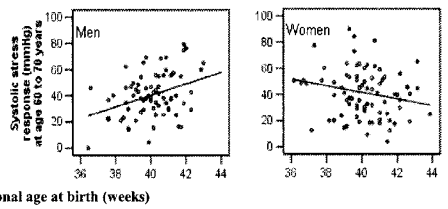


Fig. 1 Association between systolic stress response and gestational age in men (n=73) and women (n=80).

N-002

Diabetes and Coronary Heart Disease in Filipino Women: Role of Growth and Life-Course Socioeconomic Factors Claudia Langenberg[1,2], Jaclyn Bergstrom[1], Maria R. G. Araneta[1], Elisabeth Barrett-Connor[1]; *[1]Department of Family and Preventive Medicine, School of Medicine, University of California, San Diego and [2]Department of Epidemiology and Public Health, University College London Medical School, United Kingdom*

Background: Filipino women living in the US are of shorter height and have an increased risk of diabetes, compared to Caucasian American women, despite being less obese. Poor growth, particularly of the long bones of the leg, is associated with an increased risk of coronary heart disease (CHD), potentially reflecting the influence of socioeconomic disadvantage on childhood growth and adult disease. Whether poor growth and socioeconomic factors contribute to the risk of diabetes and CHD in Filipino women has not been investigated. **Methods**: Cross-sectional study of 389 Filipino women, living in San Diego County, California, with an average age (SD) of 58.7 years (9.4) at clinic visits in 1996-98, when anthropometric and laboratory measurements were taken and standardised questionnaires administered by a Tagalog-speaking nurse. A score of economic mobility (0-4) was calculated by adding childhood financial circumstances (very poor, average, well off) to thirds of adult income (<15K; 15-44.9K; =45K US dollars). Education was grouped into <13, 13-15 and =16 completed years. Diabetes was defined by 1999 WHO criteria; CHD was defined as ischemic ECG changes, Rose angina, a history of myocardial infarction or revascularization surgery. 99% of the sample were born in the Philippines. **Results:** Mean (SD) height, leg and trunk length were 1.53m (0.06), 0.71m (0.04) and 0.82m (0.03); the average BMI was 25.3 kg/m^2 (3.6). The prevalence of **diabetes** (31.3%) did not differ according to height, leg or trunk length, but decreased significantly and linearly across groups of ascending childhood financial conditions (p_{trend}=0.007), education (p_{trend}=0.001) and adult income (p_{trend}=0.001) in age adjusted analyses. Associations remained unchanged after further adjustment for weight, height, age at immigration, smoking, alcohol intake, exercise, menopausal status and HRT use, (p_{trend}=0.01 each in fully adjusted models). Further, diabetes decreased significantly and linearly across groups of ascending economic mobility; compared to Filipinas who were poor in childhood and remained in the lowest income group in adult life, the odds of diabetes decreased to 0.74 (0.28; 1.98), 0.32 (0.11; 0.94), 0.21 (0.06; 0.70) down to 0.15 (0.02; 0.92) in those who were most advantaged in childhood and in the highest income group in adult life, after adjustment for all variables mentioned above (p_{trend}=0.001). In contrast, **CHD** prevalence (22.4%) was not significantly associated with any of the socioeconomic indicators, but differed across quarters of leg, but not trunk length. Compared to those with the shortest legs, the odds of CHD decreased to 0.44 (0.21; 0.91), 0.39 (0.19; 0.81) and 0.36 (0.16; 0.78) in the tallest group, in fully adjusted models (p_{trend}=0.009). Adjusting for body size by including either BMI, total percent body fat, waist-hip ratio or waist circumference instead of weight, or excluding the 4 women not born in the Philippines had no or little effect our results. **Conclusions**: Early and adult life socioeconomic factors strongly and independently influence the prevalence of diabetes in high-risk Filipinas living in the US, a non-obese population by Western standards. Further, our results support the hypothesis that factors limiting early growth of the legs increase the risk of CHD, but not diabetes, in this comparatively short population; socioeconomic factors considered here do not seem to underlie this association. Prospective studies are warranted to validate these preliminary cross-sectional results.

N-003

Does Supplemental Nutrition in Early Life Reduce Later Risk of Cardiovascular Disease? Rameshwar Sarma K.V, Sanjay Kinra, John Cockcroft, Ian Wilkinson, Ghafoorunissa, George Davey Smith, Yoav Ben-Shlomo; *National Institute of Nutrition, Hyderabad, Andhra Pradesh, India, Department of Social Medicine, University of Bristol, Bristol, UK, Wales Heart Research Unit, University of Wales College of Medicine, Cardiff, UK, Clinical Pharmacology Unit, University of Cambridge, Cambridge, UK*

Aims: Undernutrition in early life can permanently program an individual's future risk of cardiovascular disease. Although observational studies linking size at birth to later outcomes are extensively replicated, it is still unclear whether these associations reflect the role of maternal diet in pregnancy or foetal nutrition due to placental or genetic factors. Therefore, cardiovascular disease risk among the off springs born to a cohort of chronically undernourished, non-smoking women were examined. About half of these women were resident in an area with an ongoing programme of supplemental nutrition for pregnant women and children under the age of 6 years. **Methods**: The investigators prospectively established birth cohort to assess the impact of food supplementation (500 calories, 25 grams protein, for 300 days a year) on pregnancy outcome. They selected fifteen villages with the programme and 14 villages without the programme from one area of rural south India, and recruited all women who became pregnant during 1987-1990. They collected baseline data on these women during stages of pregnancy, and their offspring during the first year of life. In the present follow up, they traced children born in this cohort and invited them to attend a locally arranged clinic. Here, they collected information on their health and lifestyle, and measured their height, weight, skin folds, waist-hip circumference, and blood pressure. The investigators also assessed the arterial stiffness (radial artery augmentation index) by the non-invasive technique of applanation tonometry, and collected fasting blood sample to measure glucose, lipids and insulin. The follow up is now complete and data is being analysed. Of the available 1,120 children at the time of study, the data was available in 964 children who formed the study group. **Results**: Preliminary analyses on 964 children (non-supplemented group: N=550, mean age=15.1 years, girls=46%; supplemented group: N=414, mean age=15.0 years, girls=47%) indicated that there were no important differences between the two groups in their height, adiposity, blood pressure, cholesterol or triglycerides. However, children in the supplemented group had lower arterial stiffness (augmentation index: 1.3 versus 5.5; P<0.001) and lower fasting serum insulin levels (17.2 mU/L versus 21.6 mU/L; P<0.001) than the control group. These differences persisted even after adjustment for age, sex, height and body mass index of the participants. **Conclusions**: Preliminary results from this controlled trial suggest that better nutrition in early life among chronically undernourished populations may confer long-germ benefits against cardiovascular risk.

N-004

Prenatal and Infant Exposure to Acetaminophen and the Risk for Wheeze and Eczema During Early Childhood Scirica CV, Rifas-Shiman SL, Gillman MW, Weiss ST, Gold DR, Litonjua AA. *Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School; Department of Ambulatory Care and Prevention, Harvard Medical School and Harvard Pilgrim Health Care, Boston, Massachusetts 02215, USA.*

Background: Several studies have reported an association between acetaminophen intake during pregnancy or early life and increased risk of asthma-related symptoms during childhood. However, because these studies did not adequately control for childhood respiratory illnesses, it remains unclear whether these findings may be confounded by the reason for taking acetaminophen (reverse causation or confounding by indication). The primary aim of this analysis was to determine whether acetaminophen intake during pregnancy or the first year of life is associated with wheeze or eczema in early childhood. **Methods:** We obtained information on

acetaminophen intake during pregnancy and the first year of life by questionnaire from the mothers of 1174 infants enrolled in Project Viva, a US cohort study of pregnant women and their offspring. The outcomes consisted of 1) asthma, defined as a diagnosis of asthma, reactive airways, or wheeze by age 2 years, 2) recurrent wheeze, defined as wheezing in both the first and second years, 3) any wheeze, defined as wheezing in either the first or the second year of life, and 4) eczema, defined as a diagnosis of eczema by age 2 years. We categorized acetaminophen intake during pregnancy as never, 1-10 times per trimester and >10 times per trimester. We categorized acetaminophen intake during the first year of life as never, 1-5 times, 6-10 times and >10 times. We performed univariate and multivariable logistic regression analyses to assess the effects of acetaminophen intake on each of the outcomes of interest, adjusting for potential confounders. **Results:** Intake of acetaminophen during pregnancy was not associated with any of the outcomes. In the univariate analyses, acetaminophen intake during infancy was associated with all forms of wheeze but not with eczema. In multivariable analyses controlling for all covariates except respiratory infections (RIs) and ear infections (EIs), intake of acetaminophen during infancy remained significantly associated with asthma and each of the wheeze outcomes. However, these associations were largely attenuated after adjusting for RIs and EIs. **Conclusions:** Our results suggest that intake of acetaminophen during pregnancy or early life is not associated with early childhood wheeze or eczema. The previously reported associations between acetaminophen intake and asthma-related symptoms may not have adequately controlled for the confounding effects of respiratory infections.

Table: Acetaminophen intake during infancy and outcomes by age 2 years. Results are reported as odds ratios (ORs) for each increase in acetaminophen intake category.

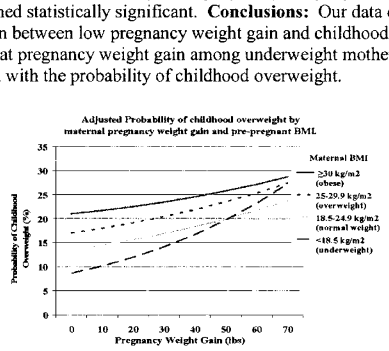
Outcome	Multivariable Model A*		Multivariable Model B**	
	OR	95% CI	OR	95% CI
Asthma	1.38	1.14, 1.67	1.18	0.97, 1.45
Recurrent wheeze	1.49	1.20, 1.85	1.15	0.89, 1.47
Any wheeze	1.27	1.10, 1.47	1.05	0.89, 1.23
Eczema	1.08	0.94, 1.25	1.09	0.94, 1.26

* Adjusted for birth weight, gender, maternal age, maternal body mass index, parental history of childhood asthma, income, exposure to passive smoking, duration of breastfeeding, number of children under 12 years of age in the home, and acetaminophen intake during pregnancy.
** Adjusted for all covariates in Model A, plus respiratory infections and ear infections.

N-005

The Association Between Pregnancy Weight Gain and Childhood Overweight is Modified by Mother's Pre-Pregnancy BMI Andrea J. Sharma, Mary E. Cogswell, Laurence M.Grummer-Strawn; Maternal and Child Nutrition Branch, Division of Nutrition and Physical Activity, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia 30341 USA.

Background: Childhood overweight increases the risk for adult obesity and maybe early onset chronic disease. Few studies have examined the association between pregnancy weight gain and subsequent childhood overweight. The objectives of the current study were to 1) examine the association between pregnancy weight gain and subsequent childhood overweight, 2) determine whether this association is modified by pre-pregnancy BMI and 3) determine whether the association is due to infant weight at birth. **Methods:** We linked data from the Pediatric and Pregnancy Nutrition Surveillance Systems for low-income 2-4-year-old children born ≥37 weeks gestation from 1995-2001 in nine states and two Indian tribes. We used logistic regression to estimate the odds and predicted probabilities of childhood overweight (body mass index-for-age-and-sex =95th percentile based on the 2000 CDC growth charts) per lb of maternal weight gain. We tested for a quadratic association between pregnancy weight gain and childhood overweight and for an interaction between pre-pregnancy BMI category and pregnancy weight gain because both maternal under- and over-nutrition may program childhood overweight. Models were adjusted for the child's age, sex, race/ethnicity, and state of birth and the mother's age, height, smoking status during pregnancy, and education. To assess mediating effects, we examined an additional model that included birth weight. **Results:** Of the 165,013 children in our study, 14.3% were overweight. The association between pregnancy weight gain and childhood overweight was both non-linear ($p<0.0001$) and modified by maternal BMI (p for statistical interaction <0.0001). The association between pregnancy weight gain and childhood overweight was strongest among children born to underweight mothers and was attenuated as maternal BMI increased (figure). Children born to underweight mothers had the lowest probability of childhood overweight until pregnancy weight gain exceeded about 45 lbs. When birth weight was added to the model, the associations between overweight and pregnancy weight gain were slightly attenuated, but remained statistically significant. **Conclusions:** Our data do not support an association between low pregnancy weight gain and childhood overweight but do suggest that pregnancy weight gain among underweight mothers is more strongly associated with the probability of childhood overweight.



N-006

Vascular Development in the Rat Placenta and the Impact of Increased Glucocorticoid Exposure Damien P. Hewitt, Peter J. Mark and Brendan J. Waddell *School of Anatomy & Human Biology, The University of Western Australia, 35 Stirling Highway Crawley, Perth, Western Australia 6009, Australia.*

Fetal glucocorticoid exposure is now recognised as a key mechanism involved in programming the adult phenotype. Thus, treatment of pregnant rats with dexamethasone reduces fetal growth and results in a range of adverse outcomes in adult offspring, including hypertension and insulin resistance (1). While these effects are likely to reflect direct actions of glucocorticoids in the fetus, indirect programming effects may also be mediated via the placenta. Indeed, dexamethasone reduces placental growth and so vascularisation and consequently maternal-fetal exchange is likely to be compromised. Recent gene deletion studies indicate that peroxisome proliferator-activated receptor-γ (PPARγ) plays a critical role in rodent development, including effects on placental vascularisation. PPARγ is a member of the nuclear hormone receptor superfamily and acts as a ligand-activated transcription factor. Previously, we have demonstrated increased expression of PPARγ in the labyrinth zone of the rat placenta near term coinciding with maximal fetal and placental growth, and prevention of this increase with dexamethasone treatment (2). In this study we investigated expression of the PPARγ target genes *mucin1* (MUC1) and vascular endothelial growth factor (VEGF), as well as vascular development in the two functionally and morphologically distinct zones of the rat placenta during normal gestation and after dexamethasone-induced fetal and placental growth restriction. Real-time RT-PCR analysis demonstrated markedly higher expression of MUC1 (3-fold; $P < 0.01$) and VEGF (4-fold; $P < 0.001$) mRNA in the labyrinth zone over the final third of pregnancy Stereological analyses of the labyrinth zone revealed that the placenta underwent a marked increase ($P < 0.001$) in the absolute volume (V) and surface area (SA) associated with both maternal blood spaces (V and SA: 5-fold) and fetal capillaries (V: 4-fold, SA: 5-fold) over the final third of pregnancy. Glucocorticoid-induced fetal and placental growth restriction (1 μg/ml dexamethasone acetate in drinking water; d13-22) was associated with reduced MUC1 (51%, $P < 0.01$) and VEGF (64%, $P < 0.001$) mRNA expression in the labyrinth zone at day 22 compared to untreated controls (term = 23 days). Stereological analyses of placentas from dexamethasone treated mothers revealed reduced ($P < 0.01$) absolute volumes and surface areas associated with maternal (V and SA: 48%) and fetal blood spaces (V: 73%, SA: 68%). Interestingly, dexamethasone specifically impaired the normal increase in fetal vessel density over the final third of pregnancy, with no effect on the density of maternal blood space measures. These data suggest that activation of PPARγ may contribute to labyrinth zone development late in pregnancy, possibly supporting vascular development via the expression of VEGF. Moreover, it appears that glucocorticoid-regulated inhibition of fetal and placental growth may be mediated, in part, via a labyrinth zone specific suppression of PPARγ and subsequent inhibition of VEGF expression. Further studies are required to determine whether PPARγ may provide a suitable therapeutic target in the treatment of fetal growth restriction.

1. Seckl JR 2004 Prenatal glucocorticoids and long-term programming. Eur J Endocrinol 151 Suppl 3:U49-62
2. Hewitt DP, Mark PJ, Waddell BJ 2004 Placental expression of PPAR isoforms in rat pregnancy and the effect of increased glucocorticoid exposure. International Congress of Endocrinology, Lisbon.

N-007

Intergenerational Effects of Fetal Programming: Potential Role of Imprinted Genes for the Inheritance of Low Birth Weight and Obesity Josep C Jimenez-Chillaron, Ryan R Faucette, Carolyn Reamer, Kristen Barry, Stephanie Ruest, Jessica Otis and Mary-Elizabeth Patti; *Research Division, Joslin Diabetes Center-Harvard Medical School, Boston, MA 02215, USA*

Many epidemiological and animal studies have demonstrated a link between low birth weight (LBW) and increased susceptibility of chronic disease during adulthood. The 'fetal programming hypothesis' has been proposed as the mechanism underlying this association, and proposes that insults or stimuli acting during critical windows of development (fetal and-or early postnatal periods) can have permanent consequences on cell/tissue structure and function. In addition, the programming phenomenon can progress to subsequent generations. This observation suggests that an epigenetic mechanism could be involved in fetal programming and its intergenerational consequences. Imprinted genes are attractive candidates for programming of adult disease since *a)* they are susceptible to epigenetic regulation, *b)* they play a key role in control of feto-placental nutrient supply and fetal growth, and *c)* a number of imprinted genes (*pref1, necdin, peg1, etc.*) have been linked to fat development and obese phenotypes. We have developed a mouse model of intrauterine growth retardation (IUGR) and LBW by restricting global food intake by 50% in pregnant ICR mice from embryonic day 12.5 (*ed*12.5) until delivery. In accord with human data, adult male mice (LBW F1 generation) develop progressive obesity, glucose intolerance and diabetes with aging. To determine whether there is a progression of (low) birth weight and adult obesity to the next generation (F2), even in the absence of altered nutrition during pregnancy, we intercrossed both female (f) and male (m) F1 adult control and LBW mice. F2 mice showed reduced birth weight ($C_f \times C_m = 1.67 \pm 0.01$g; $C_f \times LBW_m = 1.56 \pm 0.01$g, $P < 0.0001$; $LBW_f \times C_m = 1.61 \pm 0.01$g, $P < 0.01$) and developed obesity by 4 months of age (% body fat: $C_f \times C_m = 22.4 \pm 1.4\%$; $C_f \times LBW_m = 22.9 \pm 1.0\%$; $LBW_f \times C_m = 27.0 \pm 1.2\%$, $P < 0.05$; $LBW_f \times LBW_m = 27.2 \pm 0.8\%$, $P < 0.001$). We hypothesized that differential expression of key imprinted genes in placenta and adipose tissue might contribute to low birth weight and obesity respectively. We

analyzed expression of candidate imprinted genes in *ed*16.5 placentas and in epididymal fat pads from 4 month old mice, from both the F1 and F2 cohorts. Interestingly, placental expression of maternally imprinted genes (*H19, Ifg2R, Grb10, Slc22a1l*) was similar in IUGR placentas from both F1 and F2 litters as compared to controls. In contrast, placental expression of the paternally imprinted genes *ZAC1, Pref1, Necdin, Ata3 and MageL2* was significantly reduced in IUGR placentas from both F1 and F2 cohorts (30-40% reduction, all $p<0.05$). These data support the conflict hypothesis and suggest that reduced expression of growth-promoting paternally imprinted genes could account for reduced birth weight in F1 and F2 mice. Epididymal adipose expression of *necdin* and *peg1* was unaltered in all groups. In contrast, expression of *Pref1*, an inhibitor of fat development, was reduced in both F1 and F2 LBW mice (F1: 30%, $P< 0.05$; F2: C_f x LBW_m= 70%, $P< 0.05$; LBW_f x C_m= 20%; LBW_f x LBW_m= 70%). In conclusion, we demonstrate that maternal undernutrition during pregnancy reduces birth weight and increases adiposity in both 1st and 2nd generation offspring, even despite *ad lib* feeding during the second pregnancy. We propose that reduced placental expression of some paternally imprinted genes may contribute to reduced birthweight, whereas the obesity phenotype may be due, in part, to altered expression of Pref1. Taken together, these data suggest that a common regulatory (epigenetic) mechanism may underlie the intergenerational programming of birth weight and obesity.

N-008

eNOS Plays an Important Role in Uteroplacental Hemodynamics and is Required for Normal Fetal Growth Shathiyah Kulandavelu, Junwu Mu, Monique Rennie, Kathie Whiteley and S.L. Adamson; *Samuel Lunenfeld Research Inst., Mount Sinai Hospital, Depts of Physiology, Obstetrics and Gynecology & Medical Biophysics, University of Toronto, ON, M2J 3B5, Canada.*

Introduction: Intrauterine growth restriction continues to be a major cause of perinatal morbidity and mortality. The etiology is multi-factorial being both maternal and fetal in origin. Previously, we have shown that mice lacking the eNOS gene failed to adapt normally to pregnancy, in that the normal increase in maternal cardiac output was significantly blunted in late gestation. In addition, nitric oxide has been shown to be produced and active in the uteroplacental vasculature, but little is known about the role of the eNOS gene in uteroplacental hemodynamics. Thus, the purpose of this study was to examine uteroplacental hemodynamics in eNOS -/- mice. **Methods:** Pregnant control (C57Bl/6J) and eNOS -/- mice were examined while under light isoflurane anesthesia at E17.5 (n=5 in each group). Using an ultrasound biomicroscope, peak systolic velocity (PSV) and end-diastolic velocity (EDV) were measured from blood velocity waveforms obtained from the uterine and umbilical arteries and the Resistance Index (RI = (PSV-EDV)/PSV) was calculated. Umbilical artery diameter and feto-placental arterial surface area were measured from 3-dimensional images of the feto-placental arterial vasculature using micro computed tomography (n=3 fetuses). Fetal and placental weights were also recorded. **Results:** There was a large and significant elevation in the Resistance Index of the uterine artery of pregnant eNOS -/- mice (0.70 ± 0.02 vs. 0.46 ± 0.01 in controls) due to a significantly lower EDV (105 ± 27 mm/s vs. 232 ± 23 mm/s) whereas PSV was unchanged. Resistance index in the umbilical artery of eNOS -/- fetuses was only slightly higher (0.93 ± 0.01) than controls (0.90 ± 0.01) whereas there were large reductions in PSV (151 ± 5 mm/s vs.123 ± 6 mm/s) and EDV (13 ± 1 mm/s vs. 8 ± 1 mm/s)(all changes significant). PSV in the umbilical vein was also significantly lower in eNOS -/- fetuses (45 ± 4 mm/s vs. 75 ± 4 mm/s in controls). Whether low umbilical blood velocities were a cause or consequence of reduced fetal growth (0.72 ± 0.01 g vs. 0.87 ± 0.03 g in controls) is unclear. Placental weights did not differ significantly between the two groups. Nevertheless, there was a trend towards reduced umbilical artery diameter (0.44 ± 0.03 mm vs. 0.49 ± 0.02 mm) and surface area (91 ± 17mm^2 vs. 110 ± 3 mm^2) in eNOS -/- fetuses. Conclusions: Mice lacking the eNOS gene are growth-restricted and show abnormalities in uteroplacental hemodynamics similar to those observed in growth-restricted human fetuses. The increased Resistance Index in the uterine artery of eNOS -/- mice suggests that uteroplacental vascular resistance is elevated and this may contribute to fetal growth restriction in this model. These findings indicate an important role for eNOS in uteroplacental hemodynamics and fetal growth.

N-009

Chronic Maternal Overfeeding Leads to Fetal Steatosis and Metabolic Programming in the Non-Human Primate Carrie McCurdy, Jacob E. Friedman, and Kevin L. Grove; *Department of Pediatrics, University of Colorado Health Sciences Center, Denver, CO, 80045 USA* and *Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR 97006 USA*

Background: Despite the dramatic rise in the incidence of pediatric obesity, and the predicted effects of impaired glucose tolerance during pregnancy on childhood obesity, there are very few mechanistic data documenting the specific metabolic consequences of abnormal maternal insulin/glucose, leptin, or fatty acids on insulin resistance in the fetus. To address this issue we have begun a long-term project using adult Japanese Macaques subjected to a high fat diet during repeated pregnancies. **Methods**: At ages of 5-8 years old, animals were randomly assigned to either a high fat (FAT; 35% fat) or low fat (CON; 13% fat) diet with ad libitum access to diet. Animals are socially housed in groups in a large run with indoor and outdoor access and vertical play structures. A fasting blood sample was collected every two months during the diet and pregnancy for analysis of plasma glucose, insulin and leptin concentration. Twice a year the animals undergo a full IV glucose tolerance test to track insulin resistance and diabetes. Pregnancies were determined by palpation and age of fetus determined by ultrasound. Fetuses born in Year 1 (Y1) and 2 (Y2) of high fat feeding were obtained by c-section at gestational day 130 and immediately delivered to necropsy for tissue collection. Maternal and fetal blood samples were taken at the time of c-section. High stringency microarray and western blot analysis was conducted on liver and skeletal muscle samples from the fetuses. **Results**: FAT-Y2 mothers had significantly greater body weight ($p<0.05$) and had significantly higher fasting leptin and insulin during pregnancy compared with FAT-Y1 and CON. In FAT-Y2 fetuses, plasma FFA concentration were significantly higher compared with CON suggesting that fat readily crossed the placenta. Microarray analysis on liver detected 107 genes that were differentially expressed only in FAT-Y2 fetuses (45 decreased and 62 increased). Of these, 18 were related to metabolic systems; 6 were related to heat shock proteins; 19 related to cell signaling and 22 were related to transcription/translation. In the liver, many of the genes involved in insulin sensitivity were altered in FAT-Y2 fetuses but not FAT-Y1 fetuses. In addition, changes in Heat Shock Protein (HSP) family were also specific to FAT-Y2 fetuses, strongly suggestive of oxidative stress in the liver. Western blot analysis demonstrated that in liver from FAT-Y2 fetuses, there was a 60% increase in the lipogenic transcription factor SREBP1c, and a two fold increase in basal phosphorylation of p38 (stress kinase). Consistent with the elevated serum FFA in both FAT-Y1 and FAT-Y2 fetuses, liver triglycerides were also increased, with FAT-Y2 ~5-times greater than control. Furthermore, liver histology demonstrated moderate steatosis in FAT-Y1, and more severe liver steatosis in FAT-Y2. **Conclusions**: Several components of the insulin signaling cascade were affected in both FAT-Y1 and FAT-Y2 fetuses; however, the effects were more dramatic in Y2 fetuses, especially within the liver. Polymorphisms in HSP70 have been linked to susceptibility to obesity and diabetes, and diabetics have increased HSP70, likely as a result of increased oxidative stress. Elevated triglycerides and SREBP1C expression suggest that babies born from overweight mothers chronically consuming a high fat diet are born with early signs of liver steatosis and possibly liver damage. Importantly, these changes were not associated with maternal diabetes suggesting that excess fetal fatty acids may be an important determinant of fetal programming in the liver. Supported by DK060685, DK060685-S2, and the Oregon Primate Research Center Core Laboratorie

N-010

Cardiac Morphological and Functional Changes in an Intrauterine Growth-Restricted Mouse Model John CH Sun, Carole S Watson, Junwu Mu, Dawei Qu, Victor Han, and S Lee Adamson. *Departments of Physiology; Obstetrics & Gynecology; Cardiovascular Sciences Collaborative Program, University of Toronto, and Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, M5G 1X5, and the Children's Health Research Institute, London, ON, N6C 2V5, Canada.*

Objective: The insulin-like growth factors (IGFs) are essential regulators of fetal growth and their effects are modulated by the IGF binding proteins (IGFBPs). Growth-restricted fetuses and newborns in humans and animals have high circulating IGFBP-1 levels, and they have increased risk of cardiovascular diseases in adulthood. We have generated transgenic (TG) mice that have elevated levels of circulating IGFBP-1 in late gestation (E14.5-E17.5), with levels decreasing after birth. These mice are growth-restricted by 20% vs. wild-type (WT). We hypothesized that TG mice would demonstrate abnormalities in cardiac morphology and/or function. **Methods:** Anesthetized TG and WT mice at prenatal E14.5 and E17.5, and postnatal 1, 4, 8, and 24 wk were studied using an ultrasound biomicroscope (N=13-28). Blood velocity waveforms in the aorta, pulmonary artery, and left ventricle (LV) were measured using Doppler, and LV inner dimension and wall thickness were measured in diastole using M-mode ultrasound. LV dimension, wall thickness, and isovolumic relaxation time were expressed as a ratio to body weight$^{0.33}$, LV dimension, and cardiac cycle length (CL) respectively. LV weight was calculated from LV structural variables obtained from M-mode ultrasound. Myocyte size was measured at E17.5 following myocyte isolation using collagenase digestion of cardiac tissue.

Results:

% Difference between TG and WT		E14.5	E17.5	1 wk	4 wk	8 wk	24 wk
	Body weight (BW)	-14%*	-20%**	-11%**	-18%**	-16%**	-16%**
	Myocyte Volume	N/A	+29%*	N/A	N/A	N/A	N/A
LV Morphology	LV weight / BW (LVW/BW)	ns	+41%**	+12%*	ns	-21%**	-17%*
	LV dimension / $BW^{0.33}$ ($Dd/BW^{0.33}$)	ns	+17%**	ns	ns	ns	ns
	LV wall thickness / LV Dd (Wth/Dd)	ns	ns	ns	ns	-13%*	-28%**
LV Function	LV Myocardial performance index (MPI)	ns	-12%*	+13%*	+17%*	+31%**	+31%**
	LV Isovolumic relaxation time (IRT/CL)	ns	ns	ns	+20%*	+34%**	+26%*
	LV Fractional shortening (FS)	ns	ns	ns	ns	-16%*	-19%**

* $P<0.05$, ** $P<0.01$, ns = not significant.

The first phenotype observed was decreased body weight at E14.5 whereas LV morphology and function were normal. However, at E17.5, the LV dimension was enlarged in TG (increased Dd/ $BW^{0.33}$), and overall LV function was improved (decreased MPI). Myocyte hypertrophy was the main contributor to the increase in LV weight. Postnatally (1-24 wk), when circulating IGFBP-1 levels were declining, TG remained growth-restricted. By 8 and 24 wk, LV of adult TG mice exhibited wall thinning (reduced Wth/Dd), and impaired LV diastolic, systolic, and overall function

(increased IRT/CL, decreased FS and MPI respectively). **Conclusions:** We conclude that perinatal growth restriction caused by elevated IGFBP-1 alters pre- and postnatal cardiac structure and function and the alteration in IGF system in the perinatal and early postnatal period may be one of the underlying mechanisms of developmental programming of the cardiovascular system. Supported by: *Richard Ivey, OGSST, CIHR*

N-011

Chronic Hypoxia Increases Sensitivity to Noradrenaline in Small Mesenteric Arteries from Fetal Sheep in Late Gestation Sarah J Williams,[1,2] Sandra T Davidge,[2] Janna L Morrison[1] and I Caroline McMillen[1]; [1]Centre for the Early Origins of Adult Health, Physiology, School of Molecular and Biomedical Science, University of Adelaide, SA, Australia, 5005; [2]Perinatal Research Centre, Departments of Ob/Gyn and Physiology, University of Alberta, Edmonton, AB, Canada, T6G 2S2

Changes in mesenteric resistance artery function may contribute to both the short-term and long-term adverse consequences of intrauterine growth restriction (IUGR), however the effects of growth restriction on the function of fetal mesenteric resistance arteries are currently unclear. **Hypothesis:** Placental restriction and associated chronic hypoxia will increase sensitivity to noradrenaline-induced vasoconstriction in 3rd and 4th order mesenteric artery branches from late gestation fetal sheep. **Methods:** Carunclectomy was performed in non-pregnant ewes to restrict subsequent placental and fetal growth (PR). Catheters were surgically inserted in the carotid artery and jugular vein in fetuses of control and PR ewes between 110-118dGA. Blood gases were analysed regularly. Post-mortem was performed at 136-141dGA (term ~150d). Both 3rd and 4th order mesenteric arteries were mounted on a wire myograph and vasoconstriction to noradrenaline assessed. The mean gestational PO_2 was used to categorise fetuses as hypoxic (H, PO_2<17mmHg, n=5) or normoxic (N, n=7). **Results:** Hypoxic fetuses (PO_2: H, 13.5±0.9mmHg versus N, 20.1±0.8mmHg, P<0.001) were characterized by lower body weight (P<0.001) and greater relative brain weight (P<0.01). There was an increased sensitivity to noradrenaline in 4th order fetal mesenteric arteries in the H group (EC_{50}: H, 3.07 ± 0.96 mM versus N, 6.4 ± 0.24 mM, P<0.01). Furthermore, noradrenaline EC_{50} values correlated with mean gestational PO_2 when both H and N groups were combined (Figure, R^2=0.68, P<0.01). Maximum tension generation in response to noradrenaline was higher in 3rd than 4th order arteries (P<0.05), but was unaffected by hypoxia. Interestingly, the sensitivity of 3rd order arteries to noradrenaline was not modified by prevailing fetal arterial PO_2. **Conclusions:** Sensitivity to noradrenaline increased in 4th order, but not 3rd order mesenteric arteries in direct relation to the degree of fetal hypoxia experienced during late gestation, suggesting a specific effect on smaller resistance arteries. Enhanced adrenergic sensitivity in small resistance arteries following chronic fetal hypoxia may contribute to both the short- and long-term vascular consequences of IUGR.

Poster Session I

Animal Models

P1-001

Vitamin E Supplementation of Maternal Low and High Fat Diets has Divergent Programming Effects on Offspring Aortic Endothelial Function

James A Armitage, Haziq Chowdhury, Joaquim Pombo, Runa I Jensen, Lucilla Poston and Paul D Taylor; Maternal and Fetal Research Unit, Division of Reproductive Health, Endocrinology and Development, Kings' College London, London SE1 7EH, United Kingdom.

Background: We have reported reduced elasticity in offspring of rats fed a lard-rich diet during pregnancy and suckling. Here we varied the fatty acid profile and vitamin E content of maternal diets and assessed offspring aortic reactivity to phenylephrine (PE), acetylcholine (ACh), nitric oxide (NO) and passive elasticity. **Methods:** *Sprague Dawley* rats (n=10 per group) were fed *ad libitum* a control breeding diet (C, 5% w/w corn oil, 110 mg/kg Vitamin E), a control diet supplemented with vitamin E (C+, 236mg/kg), a control diet supplemented with 20% w/w lard (L, 110mg/kg vitamin E) or a control diet supplemented with vitamin E (236mg/kg) and 20% w/w lard (L+) for 10 days before mating, pregnancy and suckling. Within 48 hours of birth, litter size was standardised (4? , 4?). Pups were weaned onto a control diet (3.5%w/w corn oil at 21 days of age). At six months of age, animals were killed (CO_2). Thoracic aortic rings (2mm) were mounted on an organ bath in physiological salt solution (gassed with 95% CO_2 and 5% O_2, 37°C). Cumulative stretches in Ca^{++} free buffer (0-750 μm increase in internal diameter in 3 steps) assessed passive elasticity. Cumulative dose responses were built to PE, ACh and NO. Maximum reactivity and sensitivity (pEC_{50}) values were analysed by ANOVA. There was no effect of gender thus ? and ? data are combined and are represented as mean ± SEM. Within any row of the data table, cells with different subscripts are significantly different (P<0.05). Fishers Post hoc test was used to assess specific effects of lard and vitamin E supplementation only. **Results**: Maternal lard or vitamin E intake did not affect maximum PE reactivity. However C+ showed increased PE sensitivity compared with L+. Vitamin E supplementation of the control diet blunted endothelial dependent dilatation whereas in the lard rich diet vitamin E supplementation improved endothelial dependent dilatatation. Maternal lard or vitamin E intake did not affect offspring reactivity or sensitivity to NO. Maternal lard intake affected the passive force generated to stretch; offspring of L and L+ fed dams generated the greatest force to stretch over the cumulative stretches (RM ANOVA $P<0.006$).

	Maternal Diet			
	Control (n=16)	Control + E (n=16)	Lard (n=14)	Lard + E (n=11)
PE max (%KPSS)	84.8 ± 5.3^{a}	92.1 ± 6.1^{a}	91.2 ± 7.3^{a}	93.7 ± 8.4^{a}
PE pEC_{50} (log M)	7.9 ± 0.1^{a}	7.9 ± 0.2^{a}	8.2 ± 0.1^{a,b}	8.1 ± 0.1^{a,b}
ACh max (% relaxation)	72.1 ± 3.8^{a}	58.5 ± 4.9^{b}	72.1 ± 3.9^{a}	83.8 ± 3.3^{a}
ACh pEC_{50} (log M)	7.6 ± 0.1^{a}	7.6 ± 0.1^{a}	7.6 ± 0.1^{a,b}	7.9 ± 0.1^{b,}
NO max (% relaxation)	99.7 ± 0.2^{a}	96.3 ± 2.0^{a}	99.8 ± 0.1^{a}	99.5 ± 0.4^{a}
NO EC_{50} (log M)	8.1 ± 0.2^{a,b}	7.8 ± 0.2^{a}	8.3 ± 0.2^{b}	7.8±0.3^{a,b}

Conclusions: These data highlight the programming effects of maternal fatty acid intake during pregnancy on large artery elasticity. The observation that C+ offspring had blunted endothelial dependent dilatation and that L+ trended to improved endothelial function is novel. The data may highlight the importance of balancing fat-soluble vitamins and fat intake. Excessive vitamin E supplementation in and otherwise balanced maternal diet does not appear to confer any benefit to offspring aortic function and may induce deleterious programming effects on vascular function.

P1-002

Offspring of Rats Fed Saturated Fat Rich Diet During Pregnancy and Suckling Demonstrate Programmed Reductions in Na^+, K^+-ATPase Activity

James A Armitage, Sanjana Gupta, Joaquim Pombo, Runa I Jensen, Lucilla Poston and Paul D Taylor; Maternal and Fetal Research Unit, Division of Reproductive Health, Endocrinology and Development, Kings' College London, London SE1 7EH, United Kingdom.

Background: The offspring of rats fed a lard-rich diet during pregnancy demonstrate a phenotype akin to the metabolic syndrome associated with reduced activity of the Na^+, K^+-ATPase in whole kidney homogenates. As the Na^+, K^+-ATPase is involved in the maintenance of transmembrane ionic gradients and membrane potentials, reduced activity may result in altered organ function. Here we examine Na^+, K^+-ATPase activity in brain, retina, kidney cortex, heart and liver in offspring of rats fed diets rich in either saturated, monounsaturated or polyunsaturated fatty acids during pregnancy and suckling. **Methods:** *Sprague Dawley* rats were fed *ad libitum* a control breeding diet (5% w/w corn oil), or a control diet supplemented with 20% w/w lard, palm oil (rich in saturates, SFA), rapeseed oil (rich in monounsaturates, MUFA) or corn oil (rich in polyunsaturates, PUFA) throughout pregnancy and suckling. After 10 days, animals were mated and within 48 hours of birth, litter size was standardised (4? , 4?). Pups were weaned onto a control diet (3.5%w/w corn oil at 21 days of age). At six months of age, animals were killed (CO_2) and the cerebral frontal cortex, retina, cardiac left ventricle, renal cortex and liver rapidly dissected. Homogenised tissue was incubated with excess ATP for 5 minutes at 37°C, the reaction was quenched (ice-cold perchloric acid) and Na^+, K^+-ATPase activity estimated by colourimetric detection of PO_4 from the hydrolysis of ATP to ADP in the presence or absence of ouabain. Data (mean ± SEM) were analysed by ANOVA. **Results**: The table shows Na^+, K^+-ATPase activity (nMol PO_4 liberated.mg $protein^{-1}.hr^{-1}$). Within a given tissue, * denotes significant difference from control and diet groups with different superscript letters are significantly different from each other ($P<0.05$). Na^+, K^+-ATPase activity in kidney, brain and retina, was lower in offspring of SFA fed dams when compared with all other groups. In the kidney, offspring of Lard fed dams also demonstrated reduced activity. There was no effect in liver and heart.

	Maternal diet				
	Control (n=18)	Lard (n=11)	SFA (n=15)	MUFA (n=13)	PUFA (n=18)
Brain	167.1 ± 12.3	127.6 ± 14.9^{*,b}	124.8 ± 16.9^{*,b}	211.3 ± 15.9^{*,c}	174.1 ± 15.4^{c}
Retina	224.2 ± 18.8	250.4 ± 27.0^{a}	173.7 ± 18.4^{*,b}	270.9 ± 24.6^{a}	250.3 ± 24.9^{a}
Kidney	97.3 ± 10.1	65.1 ± 13.2^{*,c}	56.2 ± 6.8^{*,b}	98.7 ± 15.4^{a}	120.6 ± 13.3^{a}
Heart	15.2 ± 2.3	18.1 ± 3.8	11.7 ± 2.9	9.3 ± 2.2	16.5 ± 3.3
Liver	6.2 ± 1.0	5.3 ± 1.2	7.3 ± 1.3	8.0 ± 1.2	8.3 ± 1.1

Conclusions: Tissue specific programmed reduction in Na^+, K^+-ATPase activity in brain, retina and kidney is associated with exposure to SFA rather than to a fat rich diet *per se*. Offspring of MUFA fed dams demonstrated higher activity compared with controls in brain, but this is likely due to the α-linolenic acid (n-3 PUFA) component in this otherwise MUFA rich diet. Reduced Na^+, K^+-ATPase activity may result from programmed changes to membrane fatty acid profile or programmed reduction in expression or protein translation. Maternal diets rich in saturated fats may permanently programme reduced activity of vital cellular enzymes in the offspring, thus attention should be paid to saturated fat intake during pregnancy and suckling.

P1-003

Effects of Somatic Cloning on the Immune Response in Young and Adult Cattle

Chavatte-Palmer, P.[1], Nolent, F.[1], Servely, J-L[1], Renard, J.P.[1], Schwartz, I.[2]
[1]*UMR INRA-CNRS-ENVA 1198, Biologie du Développement et Reproduction,*
[2]*Virologie et Immunologie Moléculaires, 78352 Jouy en Josas cedex, France*

Background Somatic cloning in cattle is associated with important gestational abnormalities, including implantation delay during the first 2 months of pregnancy and abnormal fetal and placental growth (known as Large Offspring Syndrome, or LOS) in the third trimester. In our laboratory, between 3 and 25% of the cloned blastocysts transferred to recipient cows reach term depending on the genotype of the donor cells. About 20% of the newborns die rapidly in the first few weeks, due to various causes (cardio-vascular abnormalities and pulmonary edema, renal abnormalities, limb deformations…) that appear to be direct consequences of the LOS. We previously reported on a thymic atrophy resulting from nuclear transfer (Renard et al. 1999) and have further diagnosed distinct pathological events occurring in the infancy and adult age of clones including death due to apparently benign infections (despite treatment) and also thymic atrophy in approximately 20% of the post-mortem cases. Abnormal immune function has been reported to be possibly a consequence of fetal growth perturbation in man (Moore et al. 1999) and animal models. These observations in clones have lead us to investigate the immune function of apparently normal bovine clones. **Methods** Holstein cows housed in the same farm were used. Circulating lymphocyte population during resting state were marked and counted in 17 clones and 17 contemporary controls aged from 15 days to 5 years of age and allotted to one of 3 groups : 1 (15days-2 months, N=4 clones and N=6 controls), 2 (3-9 months, N=7 clones and N=5 controls) and 3 (20 months to 5 years, N=6 clones and N=6 controls). Clones originated from adult fibroblasts cells from 4 different genotypes distributed in the 3 groups. PMBCs were collected, marked, and counted by flow cytometry. The specific markers were CD2, CD3, CD4, CD8, CD14, CD11b, CD25, CD45RO, P46 (NK cells), γδ, PanB, MHC1 and MHC2. Results were analysed by ANOVA using SAS software after log transformation. In a second experiment, 6 clones from 3 different genotypes and 6 controls aged between 8 and 9 months were vaccinated with 10 mg Ovalbumin in alum (to evaluate the naive immune response). The antibody and cellular (TTL) responses were compared. Data were analyzed by ANOVA after log transformation. **Results** The cell subset proportions were not different between clones and controls. There was no difference between groups for antibody response to vaccination. However, T cell restimulation with specific antigens after immunization was significantly lower in clones compared to controls (P<0.05) for ovalbumin. Furthermore, non-specific stimulation with PHA was also lower in clones (P<0.05). **Discussion** These results show that lymphocyte populations are normally represented in apparently healthy clones. Bovine clones presented, however, a reduced capacity to build up a cellular immune response against a newly encountered antigen, such as ovalbumin. It remains to be determined whether these functional alterations are a result of defective reprogramming of immune functions during the cloning process or the consequence of an abnormal placental development leading to altered feto-placental interactions during pregnancy and fetal programming. Previous work by others has shown that there may be an abnormal expression of MHC1 in the placenta of bovine

clones (Hill et al. 2002), and this may well be part of the same phenomenon affecting overall immune regulation in clones.

P1-004

The Effect of Maternal Hypoxia During Late Gestation in Rats on the Cardiovascular Responses of Male Offspring to Acute Systemic Hypoxia

Andrew M. Coney and Janice M. Marshall; Department of Physiology, Division of Medical Sciences, The Medical School, University of Birmingham, B15 2TT, UK.

Introduction: Evidence from the last decade has led to the hypothesis that exposure to suboptimal conditions *in utero* can have profound health effects on a fetus in adulthood (1). Studies to date have concentrated mainly on maternal malnutrition (predominantly restricted protein intake), which can result in the long-term cardiovascular consequences of hypertension and diabetes (2). Typically, maternal malnutrition results in low birth weight and this has been used as an indicator of a developmental origin of cardiovascular disease. Chronic hypoxia *in utero* also results in low birth weight (3), however there is little data on its long-term consequences *in vivo*. These experiments investigated in rats how chronic hypoxia during the second half of gestation affected baseline cardiovascular variables and the cardiovascular response to a period of acute systemic hypoxia. **Methods:** Experiments were performed on male Wistar rats. The control group (N; n=7) were kept in normoxia throughout gestation and rearing. For the chronic hypoxia *in utero* group (CH; n=7), pregnant dams were housed in a normobaric hypoxic chamber (12% O_2) for days 10-20 of pregnancy and then returned to normoxia to give birth. Litters were housed under the same conditions as the N group. Rats were anaesthetised and instrumented for measurement of cardiovascular variables as we have previously described and periods of acute systemic hypoxia were induced by changing inspired gas to a mixture containing 8% O_2 (4). **Results:** During air breathing arterial blood pressure (ABP) and heart rate (HR) were significantly higher in CH than N rats (129±3 vs 118±4 mmHg, $P< 0.05$ and 458±18 vs 406±8 b.p.m., $P < 0.05$), but there was no difference in femoral vascular conductance (FVC) and blood flow (FBF). Arterial blood gas values evoked by acute systemic hypoxia (breathing 8% O_2) were the same in N and CH rats (PO_2 28.5±1.3 vs 31±1.5 mmHg; PCO_2 22.2±1.4 vs 21.5±1.5 mmHg). ABP during acute hypoxia was still significantly higher in CH than N rats (89±6 vs 68±4 mmHg; $P < 0.05$). Acute hypoxia evoked a tachycardia of ~25 b.p.m. in both groups. FVC, an indicator of femoral dilation was similar in both the N and CH rats, however, the maintenance of a higher ABP allowed FBF to increase during hypoxia (1.53±0.12 vs 1.76±0.14 $ml.min^{-1}$). **Summary:** The offspring of 10-20 day gestation hypoxic dams are hypertensive in adulthood under normoxic conditions and have an altered response to acute systemic hypoxia. The results of this study provide further evidence for developmental origins of cardiovascular disease and may reflect differences in the autonomic control of blood pressure and endothelial function. This work is supported by the British Heart Foundation.

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P1-005

Gene Expression Profiling in the Primate Fetal Kidney as a Framework for Evaluating Effects of 30% Maternal Nutrient Restriction (MNR) at 50% Gestation (0.5G) Laura A. Cox[1], Natalia Schlabritz-Loutsevitch[2], Robert E. Shade[1], Gene Hubbard[1], Thomas J. McDonald[2], Mark Nijland[2], Peter W. Nathanielsz[2]. [1]Southwest Foundation for Biomedical Research, San Antonio, TX, [2]University of Texas Health Science Center, San Antonio, TX.

Introduction: Preliminary studies using gene expression profiling in 0.5G fetal kidneys suggest an inhibition of cell proliferation and acceleration of cell differentiation in kidneys of fetuses of nutrient restricted pregnant baboons (MNR) compared to controls (CON). Pathway analyses of these data suggest that differentiation is accelerated in the 0.5G MNR kidney compared with CON. However, a framework of gene expression data for gestational CON at multiple time points is necessary to provide the context of altered gene expression in the 0.5G MNR kidney. We therefore performed expression profiling on CON kidneys at four gestational ages and compared these profiles with those of MNR mid-gestation kidneys. **Methods**: Fetal baboon kidneys were collected at necropsy from normal gestational controls at 0.3G (n=2), 0.5G (n=3), 0.7G (n=3) and 0.9G (n=3) and 0.5G MNR (n=3). Extracted RNA for each kidney was used to interrogate a human gene chip (Affymetrix, U133A 2.0). Data were analyzed to identify differential gene expression in the context of biological pathways (Kyoto Encyclopedia of Genes and Genomes and Gene Ontogeny Pathways) and differential gene expression in the context of hierarchical clusters using GeneSifter software. ANOVAwas used to assess differential gene expression and z-scores to assess differences in biological pathways. **Results**: Gene expression profiling and pathway analysis of 0.3G to 0.5G kidneys show a decrease in genes responsible for cell proliferation in the 0.5G kidney compared with the 0.3G kidney. Analysis of 0.5G to 0.7G kidneys shows a decrease in genes responsible for cell proliferation with an increase in cell differentiation at 0.7G compared to 0.5G. Finally, comparison of 0.7G and 0.9G shows up-regulation of genes responsible for transmembrane signaling and cell differentiation with down-regulation of cell proliferation and apoptosis at 0.9G. Pathway and cluster analysis with 0.5G MNR kidney RNA show patterns of expression similar to 0.5G CON for some genes and pathways such as the TCA cycle, similar to 0.7G CON for many others, such as apoptosis. Importantly, the 0.5G MNR kidney also exhibits patterns of expression that differ with all other time points for a few genes such as MAPK and WNT signaling. **Conclusions**: Cluster and pathway analysis of the 0.5G MNR kidney RNA samples in the context of 0.3, 0.5, 0.7, and 0.9G CON samples show that 0.5G MNR gene expression profiles are most similar to the profile of the 0.7G CON kidney. These data support the hypothesis that MNR accelerates primate kidney development. Although this study provides a framework for the 0.5G MNR primate kidney, additional analyses at multiple developmental time points are necessary to define mechanistic differences between CON and MNR kidney development.

P1-006

Gender Differences in Alterations of Gene Expression Following Maternal Nutrient Restriction (MNR) in the 0.5 Gestation Primate Kidney Laura A. Cox[1], Natalia Schlabritz-Loutsevitch[2], Robert E. Shade[1], Gene Hubbard[1],Thomas J. McDonald[2], Mark Nijland[2], Peter W. Nathanielsz[2]. [1]Southwest Foundation for Biomedical Research, San Antonio, TX, [2]University of Texas Health Science Center, San Antonio, TX.

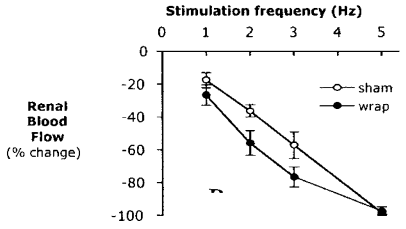
Introduction: Rodent and sheep studies show gender specific effects of MNR on fetal development. To date, similar data are not available in non-human primates. As a first step to address gender specific effects of MNR on the developing primate kidney, we performed gene expression profiling and pathway analysis on mid-gestation male and female fetal kidney mRNA samples from control mothers fed *ad libitum* (CON) and nutrient restricted mothers fed 70% of diet consumed by controls (MNR). **Methods**: Baboon kidneys were collected at necropsy from 0.5 gestation (G) male CON, female CON, male MNR, and female MNR (n=3 for each group). Extracted RNA for each kidney was used to interrogate a human gene chip (Affymetrix, U133A 2.0). Data were analyzed to identify differential gene expression, differential gene expression in the context of biological pathways (Kyoto Encyclopedia of Genes and Genomes and Gene Ontogeny Pathways) and differential gene expression in the context of hierarchical clusters using GeneSifter software. ANOVA was used to assess differential gene expression and z-scores (z) to assess differences in biological pathways. **Results**: Comparison of CON male versus CON female 0.5G kidney RNA shows 639 significantly differently expressed genes with 339 up- and 300 down-regulated in females. Furthermore, pathway analysis comparing 0.5G CON females versus CON males shows significant up-regulation in females of some pathways considered vital to kidney development such as ubiquitin cycle ($z= 2.08$), Wnt signaling ($z = 2.02$), angiotensin receptor activity ($z = 2.91$), and translation initiation ($z = 4.04$). Down-regulated pathways in 0.5G CON female compared with male include: hormone biosynthesis ($z = 4.11$), steroid metabolism ($z = 2.70$), and MAP kinase ($z = 4.11$). **Comparison** of MNR male versus MNR female samples shows 670 significantly differently expressed genes with 337 up- and 333 down-regulated in female MNR kidney RNA samples, significant up-regulation in females of cell proliferation ($z = 3.22$) and mRNA processing ($z = 3.71$). In addition, down-regulation of pathways in MNR females compared with MNR males includes: negative regulation of cell cycle ($z = 2.71$) and DNA repair ($z = 2.17$). **Conclusions**: Analysis of expression profiling data shows gender specific differences in individual genes known to be important for kidney development such as AGTR1 and genes known to be important for cell cycle regulation such as CDC27. Furthermore, these data show significant differences in pathways considered vital to kidney development such as Wnt signaling. This study is a first step towards understanding the gender specific differences in normal kidney development and gender specific differences in MNR effects on kidney development. Additional studies are necessary to correlate these gene expression date with phenotypic data.

P1-007

Increased Renal Blood Flow Response to Nerve Stimulation in Offspring of Rabbit Mothers with Chronic Hypertension Kate M Denton, Rebecca L Flower, M Wintour and Devaki Maduwegedera. *Department of Physiology, Monash University, Victoria, 3800, Australia.*

Background: Adult blood pressure is increased in offspring of rabbit mothers with chronic hypertension not of genetic origin [1]. Recently, we have also demonstrated that kidney noradrenaline content is reduced by ~20% in offspring of hypertensive mothers at birth. This suggests that in this model of programmed hypertension the fetal development of the renal sympathetic nerves is altered. The aim of this study was to determine if the physiological response to stimulation of the renal nerves was enhanced in offspring at 10 weeks of age, a time prior to the rise in blood pressure in the offspring of hypertensive mothers in this model. **Methods:** At 10 weeks of age conscious intra-arterial pressure was measured in offspring of hypertensive and normotensive mothers. Then the rabbit was anaesthetised with pentobarbitone and the left kidney exposed via a flank incision. A transonic flow probe was placed around the renal artery to measure renal blood flow and the renal nerves placed over a stimulating electrode, the nerves were section above the electrode. Following an equilibration period, renal blood flow responses to increasing frequency of electrical stimulation (1, 2, 3 and 5 Hz at 5 volts and 2 ms duration) were measured. Each frequency of stimulation was applied for 3 minutes with 5-10 minutes between stimulations for

recovery of baseline. Hypertension was induced in the mothers using a 2 kidney, 1 wrap model of renal hypertension 4 weeks prior to pregnancy. A sham-operation was performed in the normotensive rabbit mothers.



Results: Conscious mean arterial pressure was 68 ± 2 mmHg in the offspring of hypertensive mothers and 62 ± 3 mmHg in offspring of sham-operated mothers, not significantly different (P = 0.2). Baseline renal blood flow was 31 ± 4 ml/min and 25 ± 4 ml/min in the offspring of hypertensive and normotensive mothers, respectively (P = 0.1). A graded fall in renal blood flow in response to renal nerve stimulation was observed in both groups. However the renal blood flow response to electrical stimulation of the renal nerves was significantly enhanced in the offspring of hypertensive (wrap; n = 6) as compared to normotensive mothers (sham; n = 4) (see figure).**Conclusions:** The renal sympathetic nerves play an important role in the long-term control of arterial pressure. Increased renal sympathetic activity has been implicated in the pathogenesis of human essential hypertension. We have demonstrated that the physiological response to renal sympathetic nerve stimulation is enhanced in pre-hypertensive offspring of mothers with renal hypertension and thus may be a driving factor in the development of hypertension in the adult offspring. [1] Denton et al, Hypertension 41 [3 Pt 2]: 634-9, 2003.

P1-008

Dexamethasone Treatment During Pregnancy Programs a Low Nephron Number in the Spiny Mouse Hayley Dickinson[1,2], David W Walker[1], E. Marelyn Wintour[1] and Karen Moritz[2]; Department of Physiology[1] and Anatomy and Cell Biology[2] Monash University, Clayton, Australia 3800.

Background: In several species exposure of the pregnant mother to synthetic glucocorticoids early in gestation when the kidney is at a preglomerular stage of development, results in a decrease of nephron number and hypertension in the adult offspring. The spiny mouse (*Acomys Cahirinus*) is a species in which nephrogenesis is complete by the end of a relatively long (40 days) gestation (1), and in which the neonates are developmentally more similar to humans compared to other rodents. We aim to investigate the effect of treating pregnant spiny mice at a 'preglomerular' stage in kidney development with dexamethasone (dex) for 60hrs on kidney structure and blood pressure in young adult offspring. **Methods:** Dex (125μg/kg) or saline was administered to pregnant spiny mice via an osmotic mini pump for 60 hours from day 20-23 of gestation. At 20 weeks of age spiny mice were anaesthetised (2.6-2.8% isoflourane, 40 mins) and carotid artery catheters were implanted and conscious unrestrained blood pressure was recorded continuously for 1 week. All organs were weighed at post-mortem. Left kidneys were processed to resin for determination of nephron number using unbiased stereological techniques and the right kidneys were frozen for gene expression studies. **Results:** Dex treated males have a significantly lower nephron number compared to saline treated males (Table). Individual glomerular volume was significantly greater after dex treatment. Male and female spiny mice are not hypertensive at 20 weeks of age following dex treatment. There is no effect on birth weights, adult weights, and brain or kidney weights with dex treatment at 20 weeks of age. Only 60% of dex-treated males survived the anaesthesia and surgical procedures compared to 92% of females, in the saline treated group the survival of males and females was 90% and 100% respectively.

Male Stereological Analysis	Saline (n=3)	Dex (n=3)
Volume, mm^3	129.84±7.85	151.55±11.84
Glomerular number	7870±27†	6878±173
Glomerular volume, $mm^3 \times 10^{-4}$	3.58±0.16†	5.15±0.18
Total Glomerular volume, mm^3	2.82±0.13†	3.50±0.04
Corpuscle volume, $mm^3 \times 10^{-4}$	3.74±.017$^{\Psi}$	5.43±0.11
Total corpuscle volume, mm^3	2.95±0.14†	3.72±0.02

Values are means ± sem. n, No. of mice. $^{\dagger}P<0.01$, $^{\Psi}P<0.001$ between groups

Conclusions: Males from dex-treated dams show reduced nephron number and glomerular hypertrophy and appear to be more vulnerable in stressful situations than females and controls. Nevertheless, dex-treated male and female spiny mice were not hypertensive at 20 weeks of age.

1. Spiny mouse (*Acomys Cahirinus*) completes nephrogenesis before birth, Hayley Dickinson, David W. Walker, Luise Cullen-McEwen, E. Marelyn Wintour and Karen Moritz. AJP – Renal 289:273-279, 2005.

P1-009

Effect of Dietary Isoflavones on Blood Pressure and Vascular Function in the Rat [1]Gillian Douglas, [2]Giovanni Mann, [1]Lucilla Poston. [1]Maternal and Fetal Research Unit, Division of Reproductive Health, Endocrinology and Development, [2]Centre for Cardiovascular Biology and Medicine, King's College London, London, UK.

Background: Isoflavones, which are found in high concentrations in soy protein, are proposed to be cardioprotective. It has been demonstrated that in female spontaneously hypertensive rats dietary soy extracts lowered the blood pressure, and ovariectomized rats fed a diet rich in isoflavones have improved endothelial function. The aim of this study was to investigate the cardiovascular effects of life-long exposure to dietary soy derived isoflavones in male and female rats. **Methods**: Male and Female Wistar rats were fed either a low isoflavone (LI, 313 mg/Kg) or high isoflavone (HI, 9.9 mg/Kg) containing diet (SDS, UK) which were otherwise identical in nutrient composition, for 10 days prior to mating and throughout pregnancy and suckling. Offspring were weaned onto and maintained on the same diet as their dam and sire. At six months of age, animals were implanted with a radio-telemetric cardiovascular monitoring device (DSI, USA). Data were collected for 10 seconds every 5 minutes over a one week period and 12hour day- and night-time averages calculated. Animals were killed and third order mesenteric arteries dissected. Arteries were mounted on a small vessel myograph (DMT, Denmark) and dose response curves obtained to the constrictors noradrenaline (0.1μM-10μM) and endothelin-1 (0.1nM-30nM) and to the dilators acetylcholine (1nm-10μM) and nitric oxide (0.1μM–10mM). Noradrenaline was used to pre-constrict vessels prior to assessment of dilator function. Mesenteric arteries were also mounted on a pressure myograph (Living Systems, USA). Distensibility was assessed from the pressure (20-100mmHg)-internal diameter curve obtained in calcium free medium. Thoracic aorta was dissected and mounted in an organ bath (DMT, Denmark) dose response curves to the constrictor phenylephrine (3nM-10μM) and the dilators acetylcholine (3nM-10μM) and nitric oxide (0.1μM-0.3mM) obtained. Phenylephrine was used to pre-constrict the vessels prior to assessment of dilator function. Data were assessed using repeated measure (RM) ANOVA and one-way ANOVA, $P<0.05$ was considered significant. When results for males and females did not differ, data were combined. Data are presented as the mean ± SEM. **Results**: Mesenteric arteries from male and female animals fed the LI diet demonstrated decreased sensitivity to acetylcholine compared with animals fed the HI diet (pEC_{50} LI 6.77 ± 0.1, n=16 *versus* HI, 7.31 ± 0.1, n=20, P<0.05). Mesenteric arteries from female but not male LI fed rats were less distensible than those of HI fed females (n=6 per group, $P<0.05$, RM ANOVA). Feeding a HI diet compared to a LI diet did not alter responses in mesenteric arteries to noradrenaline, endothelin-1 or nitric oxide or aortic responses to phenylephrine, acetylcholine or nitric oxide in arteries from male and female rats. There was no difference in systolic (LI 121 ± 1 mmHg n=16, HI 118± 1, n=20, males and females combined) or diastolic blood pressure (LI 92 ± 0.7 mmHg n=16, HI 90 ± 1, n=20), heart rate (LI 383 ± 4 beats/min n=16, HI 383 ± 4, n=20) or activity (LI 3.68 ± 0.12 arbitrary units n=16, HI 3.67 ± 0.198, n=20) between the two dietary groups. **Conclusions**: In conclusion life-long consumption of a diet rich in isoflavones increased mesenteric arterial distensibility in female rats and enhance endothelial dependent dilation in male and female rats, but has no effect on aortic function, blood pressure or heart rate at 6 months of age. Further studies will determine whether these differences are acquired in early life or as consequence of adult dietary intake.

P1-010

Impact of Early Gestation and Early Post-natal Undernutrition on Adult Coronary Artery Vascular Reactivity in Sheep OA Khan[1,2], C Torrens[1], DE Noakes[3], L Poston[4], SK Ohri[2], MA Hanson[1] & LR Green[1], [1]Centre for Developmental Origins of Health & Disease, University of Southampton, SO16 5YA; [2]Wessex Cardiothoracic Unit, Southampton General Hospital, SO16 6YD; [3]Department of Veterinary Reproduction, Royal Veterinary College, UK, AL9 7TA; [4]Division of Reproductive Health, Endocrinology & Development, GKT Hospitals, London, SE1 7EH.

Background: Epidemiological studies highlight the importance of birth weight and early post-natal growth as risk factors for the development of coronary heart disease in later life. To date, the mechanisms underlying these associations remain unclear. We investigated the effect of moderate early gestation and early post-natal nutrient restriction on the vascular reactivity of coronary arteries in sheep. **Methods:** Welsh mountain ewes (UK Animals (Scientific Procedures) Act 1986) were mated and assigned to three dietary groups: **A)** 100% of total nutritional requirements (Group CC, n=10); **B)** 50 % of total nutritional requirements during the first 30 days of gestation, followed by 100% of total nutritional requirements thereafter (Group UC, n=9); **C)** 50 % nutritional restriction during the first 30 days of gestation; followed by restriction in the diet of their offspring between 12-25 weeks post-natally, designed to produce a 15% reduction in growth trajectory (Group UU, n=7). Following these restrictions, the offspring were fed ad-libitum. The male offspring were then sacrificed at 2.5 years, the distal anterior interventricular artery harvested, mounted on a wire myograph and the vasoreactivity of the vessels measured. Data are expressed as mean ± S.E.M. Intergroup comparison were made by ANOVA and Bonferroni *post hoc* tests

Results:

	Group CC	Group UC	Group UU
Maximal Response			
Acetylcholine	163.6 ± 55.8	157.2 ± 50.7	208.6 ± 55.3
U46619	93.1 ± 31.7	88.5 ± 29.7	68.9 ± 22.7
Endothelin	185.5 ± 40.5	163.2 ± 19.1	177.7 ± 20.4
Bradykinin	84.0 ± 3.29	83.6 ± 4.59	66.3 ± 5.44*
Adenosine	50.9 ± 9.48	66.9 ± 11.6	53.2 ± 10.1
pEC_{50} (-log M)			
Acetylcholine	5.69 ±0.10	6.36 ± 0.21*	5.83 ± 0.09
U46619	7.20 ± 0.14	7.02 ± 0.11	7.11 ± 0.14
Endothelin	9.20 ± 0.20	8.85 ± 0.14	9.02 ± 0.20
Bradykinin	8.49 ± 0.33	7.94 ± 0.34	7.77 ± 0.22
Adenosine	5.59 ±0.20	5.58 ± 0.28	5.26 ± 0.19

Sensitivity to acetylcholine was significantly increased in UC compared to control ($p<0.05$*). Maximal response to bradykinin was lower in UU compared to control ($p<0.05$*). **Conclusions:** Undernutrition in early gestation and early post natal life alters coronary artery vasoreactivity in adulthood. The nature of this vascular dysfunction depends on the timing of the nutritional insult in development.
This work was supported by the British Heart Foundation and HOPE.

P1-011

The Impact of Perinatal Dietary Alterations on Offspring Cardiovascular Function in a Genetic Mouse Model
Brian Knight[1,2], Nana Sun[1], Craig Pennell[1,3], Stephen Lye[1,2]. [1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada [2]Obs/Gyn and Physiology, University of Toronto, Toronto, ON, Canada [3]School of Women's and Infants' Health, The University of Western Australia, Perth, Australia.

Objective: The association between sub-optimal intrauterine and postnatal environments and the development of adult cardiovascular disease is variable suggesting that genotype may contribute to eventual outcome. Chromosome substitution stains of mice allow the genetic dissection of complex traits; however, to date, they have not been utilized to evaluate the developmental origins of adult disease. Gene-environment interaction studies require accurate description of phenotype and to utilise mice for these studies requires detailed assessment of cardiac function. The objective of this study was to characterize cardiac function through ultrasound biomicroscopy (UBM) and peripheral blood pressure analysis in offspring of mothers subject to maternal dietary restriction (DR) during pregnancy and a high fat postnatal diet. **Methods:** Six A/J pregnant mice underwent 30% DR from 6.5 days (d) until 18.5d of gestation: a further 6 mice in each strain served as controls on an *ad libitum* diet. Mice were allowed to deliver spontaneously and their offspring were subjected to either a high fat/sucrose diet (HF) (n= 23) or an *ad libitum* control diet (n=25) at weaning. Blood pressure (BP) and heart rate were assessed using a tail cuff measurement system at 3 and 6 months of age. Cardiac function was assessed using UBM echocardiography at 6 months of age and the following echocardiographic parameters were determined: ejection time; fractional shortening; left ventricle internal diameter (LVID) during systole and diastole; E:A ratio (E-wave = peak velocity of early passive ventricle filling, A-wave = peak velocity during atrial contraction); and Tei index (TI) ((isovolemic contraction time+isovolemic relaxation time)/ejection time), an index of myocardial performance. **Results:** At 3 months of age, mice exposed to maternal DR during pregnancy had *lower* mean arterial pressure (MAP) than controls (-22mmHg, $p<0.05$) whereas by 6 months of age they had significantly *higher* MAP than controls (+26mmHg, $p<0.05$). There was no effect of modification of postnatal nutrition on MAP at 3 or 6 months of age. Echocardiography at 6 months of age showed no effect of maternal DR during pregnancy on ejection time, fractional shortening, LVID during systole or diastole, E:A ratio or TI index unless the maternal DR was combined with a postnatal HF diet. When the two adverse environments were combined there was evidence of significant cardiac dysfunction as demonstrated by: a 14% decrease in LVID during diastole, an 11% decrease in ejection time, a 20% decrease in E:A ratio, and a 39% increase in TI (all $p<0.05$). **Conclusion:** In this study we have demonstrated that in A/J mice maternal DR during pregnancy results in hypertension in offspring by 6 months of age and this relationship is not modified by postnatal nutrition. In contrast, maternal DR during pregnancy had little demonstrable effect on cardiac function assessed by UBM echocardiography unless it was combined with a postnatal HF diet which resulted in significant cardiac dysfunction. UBM is a novel technique which can be used to accurately describe cardiac phenotype in murine models of developmental origins of adult disease and may be a useful technique to define phenotype in chromosomal substituted strains in gene:environment interaction studies.

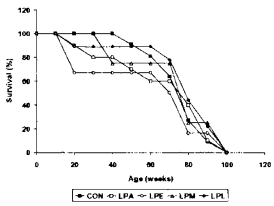
P1-012

Survival of Male Rats is Unaltered by Exposure to a Maternal Low Protein Diet During Specific Periods of Intrauterine Development
Simon C. Langley-Evans and Dean V. Sculley. School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough, LE12 5RD, United Kingdom.

Background: Exposure of the rat fetus to a maternal low protein (MLP) diet is known to programme a range of adverse outcomes in later life, including hypertension and renal dysfunction. Small scale studies of longevity in rats exposed to MLP feeding throughout fetal development have suggested that survival may be compromised by early life undernutrition. Although different experiments have independently indicated that longevity is programmed in a sex-specific manner, data are conflicting in terms of whether it is male or female offspring that exhibit a shorter lifespan following intrauterine exposure to MLP . In this much larger study, the impact of exposure to MLP during specific periods of gestation, in addition to throughout fetal development, was evaluated in male offspring. **Methods:** 57 virgin female Wistar rats were mated and allocated to be fed control or low protein diets in pregnancy. MLP feeding was targeted at d0-22 (LPA), d0-7 (LPE), d8-14 (LPM) or d15-22 (LPL) gestation. A total of 44 rats proceeded to full term gestation and produced healthy litters (CON: n=10, LPA n=9, LPE n=8, LPM n=7, LPL n=10). All litters were culled to 8 pups (4 males and 4 females) per litter at birth and then one male and one female animal from each litter were culled at 4 weeks, 9 months and 18 months of age for tissue collection. Remaining male animals were housed in pairs and allowed to live a full lifespan. Animals were euthanased if showing major signs of distress or ill-health. Survival data were analysed using Cox Regression. **Results:** As shown in the table below, mean age at death was unaltered by exposure to the MLP diet at any stage of development, although rats of the LPE group had a mean lifespan that was 24% shorter than seen in the control group. The figure shows the survival distributions for the animals in all groups, which were not significantly influenced by the maternal diet.

Maternal dietary group	CON	LPA	LPE	LPM	LPL
Mean age at death (weeks)	71 ± 14	63 ± 27	54 ± 32	68 ± 21	72 ± 24
Survival Exp (B)	1.00	0.88	1.96	1.20	0.60
95% Confidence Intervals		0.37-2.07	0.71-5.43	0.47-3.04	0.26-1.39

Age at death is presented as mean ± SEM. There were no significant effects of maternal diet on overall lifespan or the survival distributions of the 5 groups.



Conclusions: This is the largest scale study of longevity in rats following prenatal nutrient restriction and the first study to consider the importance of critical periods in development. Contrary to earlier reports, the longevity of male rats was unaltered by prenatal protein restriction. This suggests that mechanisms of ageing are not significantly influenced by early life nutrition.

P1-013

Determinants of Fetal Growth in Rats Fed Diets of Varying Protein Content
Simon C. Langley-Evans and Sarah McMullen. School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough, LE12 5RD, United Kingdom.

Background: The feeding of a maternal low protein (MLP) diet in rat pregnancy is known to programme a number of adverse physiological and metabolic states in the resulting offspring. Most studies of the effects of MLP feeding have noted that such changes occur independently of fetal growth changes, as MLP exposed animals are generally of normal weight at birth. However, previous studies have shown that MLP exposed fetuses may grow more rapidly in the earlier stages of fetal development and suffer a late gestation growth retardation that may selectively impact upon the truncal organs. As rats are litter-bearing animals capable of producing up to 20 offspring in a single litter there are a number of factors that may determine the nutrient supply that reaches each individual fetus. These factors may include hormonal interactions between fetuses in the litter. The objective of this study was to assess the interaction of maternal diet with other intrauterine variables in determining the growth of rat fetuses. **Methods:** 32 virgin female Wistar rats were mated and allocated to be fed control (n=16) or low protein (n=16) diets in pregnancy. MLP feeding was targeted at the full period of gestation. Pregnant animals were culled at either day 18 or day 20 of gestation. The fetuses and placentas were carefully dissected and weighed to the nearest milligram. All fetuses were sexed on the basis of genito-anal distance, and their position in the uterine horn, the sex of the neighbouring fetuses in the horn and the total number of viable and reabsorbed fetuses in the litter were noted. **Results:** Fetal weight increased significantly between day 18 and day 20 gestation. As shown in the table below, the gestational increase in weight was significantly influenced by the maternal diet. MLP exposed fetuses tended to be 10-12% larger than controls at day 18, but by day 20 were of similar weight. Position in the uterine horn did not significantly alter fetal weight. Male fetuses were larger than females at both points in gestation. The impact of the sex of neighbouring fetuses did not achieve statistical significance (P=0.061). However, male fetuses tended to be larger when between two females and smaller if between a female and another male. Female fetuses tended to be larger if between a female and a male and smaller when between two males.

FETAL WEIGHT (g)					
	Gestational Age	18 days		20 days	
	Maternal Diet	CON	MLP	CON	MLP

Male		1.361 ± 0.073	1.502 ± 0.066	3.710 ± 0.065	3.733 ± 0.081
Female		1.279 ± 0.071	1.429 ± 0.064	3.633 ± 0.068	3.438 ± 0.085
Maternal Diet *P*	0.547				
Fetal Sex *P*	0.024				
Gestation *P*	<0.001				
Diet x Gestation *P*	0.043				

Data are shown as mean ± SEM. *P* values shown in table are after adjustment for litter size ($P<0.001$), position in horn (NS), and sex of neighbouring fetuses ($P=0.061$).

Conclusions: These data are consistent with previous findings that prenatal protein restriction impacts upon fetal rat growth in the later stages of gestation. Fetal growth in the rat is clearly subject to a number of influences that may include transfer of sex hormones between the fetuses in a litter.

P1-014

Intrauterine Programming of Mesenchymal Stem Cell Activity S. A. Lanham, C. Roberts, C. Cooper, and R. O. C. Oreffo; Bone and Joint Research Group, Developmental Origins of Health and Disease, University of Southampton, Southampton, SO16 6YD, UK

Background. Epidemiological studies suggest skeletal growth is programmed during intrauterine and early postnatal life. We hypothesize that age-related decrease in bone mass has, in part, a fetal origin and are investigating this using a rat model of maternal protein insufficiency. **Methods.** Dams received either 18% (control) or 9% (low protein) diet during pregnancy, and the offspring (n=194) studied at selected time points (4, 8, 12, 16, 20, 47 weeks). **Results.** Maternal weight was unaffected by diet during pregnancy. Control diet mothers had significantly larger litters. Male offspring in the restricted diet group showed a modulated growth trajectory only between 4 to 8 weeks of age. In contrast, the growth trajectory was significantly reduced in female offspring in the restricted group at all time point studied from 4 weeks to 47 weeks. Total number of colony forming units (indicative of colony forming efficiency and proliferation potential of mesenchymal stem cells) increased with age and was similar with both diets. The total number of alkaline phosphatase (AP) positive CFU (indicative of osteogenic potential and differentiation) increased with age with both diets, but to a lesser extent in the control diet. The percentage of AP positive CFU dropped from 45% to 15% over 20 weeks with the control diet, but remained constant at 30% with the restricted diet. These results were observed in both sexes and were unaffected by the addition of Vitamin D3, IGF-1, or Growth Hormone. However, specific AP activity increased over 20 weeks in the control group, but decreased in females with the restricted diet. Total protein levels followed the same trend. Specific AP activity was significantly higher with the restricted diet in both males and females at 4 weeks, and females at 8 week. However, levels were significantly higher with the control diet in females at 16 and 20 weeks, and males at 12 weeks. No differences were found in collagen production by CFU between the diet groups. Analysis of serum showed a significantly lower level of IGF-1 in female offspring in the restricted diet group at 4 weeks of age. In addition, osteocalcin levels were significantly lower in both males and females in the restricted diet group at 4 weeks of age.
Conclusions. These data indicate that a low protein diet *in utero* affects both mesenchymal stem cell activity and osteogenic potential in the offspring. In particular, female offspring in the restricted group showed evidence of enhanced stem cell activity and functional activity at an early time point (4 weeks of age). These results further support the need to understand the key role of the nutritional environment in early development on programming of skeletal development with implicit consequences in later life.

P1-015

Intrauterine Programming of Skeletal Development S. A. Lanham[1], C. Roberts[1], J. Burford[2], T. M. Skerry [3], P. Taylor[4], L. R. Green [5], M. A. Hanson [5], C. Cooper[1], and R. O. C. Oreffo[1]. [1]Bone and Joint Research Group, Developmental Origins of Health and Disease, University of Southampton, Southampton, SO16 6YD, UK; [2]The Royal Veterinary College, London, UK; [3] Academic Unit of Bone Biology, University of Sheffield, UK; [4]Osteoporosis Centre, Southampton General Hospital, UK; [5]Centre for Developmental Origins of Health and Disease, University of Southampton, UK

Background. Epidemiological studies suggest skeletal growth is influenced by the intrauterine and early postnatal environments. We hypothesize that age-related decrease in bone mass has, in part, a fetal origin and are investigating this using an ovine model. **Methods.** Rams were exposed to a global nutrient restricted maternal diet *in utero* (50% total nutrient requirements, 0-30 days gestation), UC, n=6)), postnatal nutrient challenge to reduce body weight to 85% target weight from weaning (12 weeks) to 25 weeks postnatal age (CU, n=5), or both (UU, n=7). Control diet (CC, n=11). All measurements on offspring were taken at 2.5 years. **Results.** Anthropometric measurements of femurs showed no significant differences between groups, however all measurements showed a trend, compared to the control group, for the mean to be lower in the CU group, higher in the UU group, and similar in the UC group. When analysed by pQCT, femurs showed no significant differences between the groups, although the same trends were again observed, this time with cortical and trabecular Bone Mineral Density (BMD), Bone Mineral Content (BMC), cortical thickness and trabecular area. These remained after normalising to body mass. Examination of vertebrae (CC n=2, UC n=2, and UU n=2, analysed in triplicate) using DXA showed BMD in the UC group was significantly lower than CC, whereas the UU group was similar to CC. No differences were found in BMC. Significance was lost when results were normalised to mass. In a further study to examine if maternal nutrient restriction *before conception* had any effect, vertebrae from control (n=3) and male offspring from sheep exposed to a 50% nutritional restriction for 30 days before conception (n=8) were tested in duplicate. No significant differences were found with BMD or BMC. **Conclusions.** These results suggest that reduced nutrition in early gestation or early postnatal life negatively impacts on bone structure while bone formation is unaltered, or enhanced, with combined pre and postnatal nutrient restriction indicating that minimal mismatch between the pre- and postnatal nutrient environment is beneficial to skeletal development. These results indicate the key role of the nutritional environment on programming of skeletal development with implicit consequences in later life.

P1-016

Intrauterine Programming of Bone Structure C. Roberts[1], M. J. Perry[2], S. A. Lanham[1], C. Cooper[1], and R. O. C. Oreffo[1]. [1] Bone and Joint Research Group, Developmental Origins of Health and Disease, University of Southampton, Southampton, SO16 6YD, UK; [2] Department of Anatomy, University of Bristol, UK

Background. Epidemiological studies suggest skeletal growth is programmed during intrauterine and early postnatal life. We hypothesize that age-related decrease in bone mass has, in part, a fetal origin and are investigating this using a rat model of maternal protein insufficiency. **Methods.** Dams received either 18% (control) or 9% (low protein) diet during pregnancy, and the offspring (n=194) studied at selected time points (4, 8, 12, 16, 20, 47 weeks). **Results.** Maternal weight was unaffected by diet during pregnancy. Control diet mothers had significantly larger litters. At delivery, the control mean pup mass was significantly less compared to restricted diet pups. In contrast, the reverse was observed at 4 weeks indicating modulation of growth trajectory. In males, this lag in the restricted diet group disappeared by 8 weeks, but remained in females at all time points examined (4 - 47 weeks). Femora from males in the restricted diet group were longer than controls at 47 weeks of age. Restricted diet group females had shorter tibiae at 12 and 20 weeks of age. In the restricted diet groups, when normalized to mass, females showed longer tibiae and femora per unit mass at 12 weeks, whereas males showed longer femora per unit mass at 12 weeks and shorter at 20 weeks. An initial pilot study using a PIXImus bone densitometer showed no differences between the diet groups in bone mineral content (BMC) or bone mineral density (BMD) in the skull, vertebrae, femur, or tibia in the animals tested (2 males + 2 females per diet group per time point). However, there were interesting growth observations. BMC and BMD continually increased up to the 47 weeks time point in all bones studied. Males and females had similar skull BMC and BMD up to 12 weeks of age. From 16 to 47 weeks the males showed greater skull BMC and BMD than females. A similar result was seen for vertebral BMC, however, vertebral BMD was the same in both males and females. For femoral and tibial BMC, male and female graphs diverged after 8 weeks of age, although femoral and tibial BMD were similar between both sexes. **Conclusions:** These studies present evidence of site specific, and sex specific, modulation of skeletal development under the conditions studied. The attenuation of female development was seen in terms of BMC rather than BMD as observed in human epidemiological studies and our own aged rat studies (Roach et al., J. Histochem. Cytochem. 2003;373-383). These results further support the need to understand the key role of the nutritional environment in early development on programming of skeletal development with implicit consequences in later life.

P1-017

Fetal Target Organs of Estrogens and Hormonal Imprinting; Use of a Transgenic Estrogen Reporter Mouse Josephine G. Lemmen[1], Herdis Stavnsgaard[1], Paul van der Saag[2], Per Guldberg[3], Anne Grete Byskov[1] [1] Laboratory of Reproductive Biology, Juliane Marie Centre, Rigshospitalet, Copenhagen, Denmark. [2] Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Utrecht, The Netherlands. [3] Danish Cancer Institute, Copenhagen, Denmark

Background: Fertility problems and cancer incidence in estrogen target organs are increasing in the general population. One of the suspected causes is exposure to environmental compounds with estrogenic properties. This is partly based on experience from *in utero* exposure to the synthetic estrogen diethylstilbestrol (DES) in humans and rodents. More specifically, DES exposure leads to a number of disorders in sex organs of male and female offspring that resemble those which are found with increasing frequency in the general population, supporting the importance of *in utero* exposure to environmental compounds with estrogenic activity in the etiology of these diseases. The aim of this project is to enhance the understanding of the role of epigenetic changes in the association between exposures to environmental estrogenic chemicals *in utero* and subsequent cancer risk. **Methods**: We developed an *in vivo* mouse model to detect estrogenic activity of suspected environmental estrogens. These "estrogen-reporter mice" are transgenic, carrying a luciferase reporter gene coupled to an estrogen responsive promoter. The reporter gene activation can be seen as one of the first endpoints of an activated estrogen receptor-signaling pathway, as it requires ligand binding, coactivator recruitment and transactivation. These mice were exposed *in utero* to either a single high dose of estradiol-dipropionate (EP) or oil on embryonic day (E)13.5 or daily doses of DES or oil on E10-13.5. Organs were isolated at E14.5 or 21 days postpartum (p.p) for luciferase activity measurements and/or DNA/protein isolation. Methylation status of the promoter has been shown being one of the possible mechanisms behind hormonal imprinting of genes. Therefore, methylation specific PCR on promoters of estrogen target genes was carried out on bisulphite treated DNA isolated from the 21 p.p. mice. **Results**: Here we used the model to determine the short and long term effects of *in utero* exposure to the estrogens DES and EP on reporter gene expression and gene methylation/expression. We show that a broad range of

organs (ovary, testis, reproductive tract, liver, kidney, adrenal, heart, lung and mammary glands) are targets of EP 24 hours after exposure. There is no effect of either a single dose of EP or daily doses of DES on reporter gene expression at 21 days p.p. as compared to oil control animals. Interestingly, we show that the promoter of estrogen receptor alpha (ERa) is partly methylated in the mammary glands of mice exposed to a single dose of EP *in utero*, whereas in oil exposed animals all mammary glands were unmethylated. In contrast, in DNA from uteri of both oil and EP exposed animals the ERa-promoter was completely unmethylated, suggesting a tissue specific effect. **Conclusion**: The transgenic estrogen reporter mice are a useful tool to detect activation of estrogen receptors during fetal life, but no long-term changes in reporter gene activity could be detected. In contrast, a permanent change in methylation of the promoter of ERa was detected in the mammary glands of mice exposed *in utero* to a single dose of EP. Promoter methylation of target genes could be a mechanism behind the long term effects of *in utero* exposure to estrogens.

P1-018

Analysis of Cardiac Teratogenicity of Lithium in the Mouse Embryo using Doppler Echocardiography: One time Exposure at ED 6.75 Results in Abnormal Cardiac Function and Dysmorphogenesis Shyam M. Manisastry, Mingda Han, Maria Serrano, James C. Huhta, and Kersti K. Linask; *Department of Pediatrics, USF-Children's Research Institute, St. Petersburg, FL 33701*

Background: Pregnancy poses a major problem in the treatment of bipolar disorder with the drug lithium (Li). Li crosses the placental barrier during pregnancy and reaches similar levels in the fetus as in the mother. In humans Li has been implicated in anomalies of the developing heart, including Ebstein's anomaly, as well as brain damage, stillbirth, and premature birth. Although the cardiac teratogenic risk is considered real and the most prevalent among lithium-linked anomalies, clinically it appears relatively infrequently (0.05%-1%) with prenatal exposure to Li. Animal studies with rodent models used gestational stages after a tubular heart has already formed, i.e., embryonic day (ED) 8 or older, and demonstrate various defects with lithium exposure, but few cardiac related. It remains controversial whether lithium affects heart development. Our previous studies indicated that beta-catenin, a downstream target of Li/Wnt signaling, is important in cardiac compartment formation and cardiomyocyte differentiation. To assess Li effects on heart formation, we exposed mouse embryos to Li much earlier than in previous studies, i.e., during cardiac specification stages. **Methods**: After determining the effective teratogenic dose, pregnant C57Bl6/Jfemale mice received a single intraperitoneal (IP) injection (100 microliters of 6.5 mg/ml) of lithium on ED 6.75. One hour after IP injection, spectroscopic analysis for lithium in the embryos *in utero* shows ~ 33 fold increase in lithium concentrations (<0.2 micromolar in control to 6.5 micromolar in Li-exposed embryos). Embryos within a litter are seen to be at varying developmental stages and littermates displayed variability in Li absorption. Therefore a variability in results was expected. Doppler echocardiography for embryonic mouse heart function was performed between ED 13.5 until ED 16.5. Same stage control litters exposed to NaCl were monitored for comparison. Pregnant females then were euthanatized and fetuses removed. Hearts were isolated and fixed in 4% paraformaldehyde, embedded, and sectioned for morphological changes. **Results:** One time injection of lithium during early gestation between ED 6.0 to 6.5 resulted in embryonic resorptions. Exposure to Li between ED 6.75 to ED 7 results in cardiac defects: Doppler echocardiography of 5 litters (22 embryos monitored) revealed abnormal echo patterns in 82 % of the embryos (18/22), correlating with defective atrioventricular valve development and conotruncal defects. Arrythmia was also observed. Lithium exposed embryos often were much delayed in development, as compared to control embryos. This developmental delay was evident in cardiac function by the absence of an E wave on ED 14.5. Right atrium, tricuspid valve and outflow tract displayed morphologic abnormalities. A single exposure one day later on ED 7.75 no longer resulted in heart defects, but caused more posterior defects, as limb abnormalities seen in 3 out of 9 embryos. Echocardiographic patterns of control, NaCl exposed, embryos were all normal. **Conclusions:** Adverse effects of lithium on mouse heart development occur after a one time administration of Li. This effect is limited to a narrow developmental window associated with cardiac specification between ED 6.75-ED 7. During human embryonic development this sensitive window would represent days 16-21 of gestation. Earlier exposure to a peak dose of lithium results in embryonic lethality. Later exposure no longer affects the heart. Thus the reported clinical cases of heart defects associated with lithium exposure apparently represent those embryos exposed to a peak dose during a sensitive developmental window during which valve and outflow tract regions are specified in association with the secondary heart field.

P1-019

Effect of Intrauterine Environment on Vascular Molecular Pathways and Proteomic Profile Monica Longo, Fangxian. Lu, Phyllis K. Orise, Gary D.V. Hankins, Garland D. Anderson, and George R. Saade; *Dept. of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas. 77555 USA*

Background: We have previously shown that vascular function in adult mice offspring heterozygous for a non-functioning NOS3 allele depends on whether they developed in a normal mother or one lacking a functional NOS3. Our cross-breeding and embryo-transfer experiments confirmed the role of the uterine environment in the fetal programming of adult vascular function. **Objective:** To investigate potential mechanisms responsible for the altered vascular responses in later life seen in the previously characterized animal model of fetal programming induced by a genetically-dependent unfavorable uterine environment. Proteomics will be used to identify protein biomarkers and real-time RT-PCR to evaluate the expression of specific gene as cyclooxygenases (COX1 & 2) and nitric oxide synthase (NOS1 & 2) involved in the altered vascular responses seen in these animal model in later life. **Methods:** Homozygous NOS3 knockout (C57BL/6J-$NOS3^{-/-KO}$) and wild type mice ($NOS3^{+/+WT}$) were cross-bred producing maternally- ($NOS3^{+pat/-mat}$) and paternally-derived heterozygous NOS3 ($NOS3^{+mat/-pat}$) litters. Female offspring at 8-10 weeks of age were sacrificed (n=4-5/group). Aorta, kidney and liver were isolated, total RNA extracted and real-time RT-PCR using TaqMan probe for the target genes was performed. NOS1, NOS2, COX1 and COX2 mRNA expression were normalized to 18s rRNA. One-way ANOVA and Bonferrroni post-hoc test was used for statistical analysis. At the same time from the same mice serum was prefractionated at different pH and SELDI technique (Surface-Enhanced Laser-Desorption Ionization) was used to detect differential protein biomarkers. Clustering algorithm and Principal Component Analysis (PCA) were used to identify. **Results:** Gene expression: in the aorta NOS1, NOS2 as wells as COX1 mRNA expressions were significantly lower in $NOS3^{-/-KO}$ and $NOS3^{+pat/-mat}$ female mice compared with $NOS3^{+/+WT}$ ($p<0.005$) while COX2 expression was not different. In the kidney NOS1, NOS2, COX1 and COX2 expression did not differ between $NOS3^{+pat/-mat}$ and $NOS3^{+mat/-pat}$ female mice. Similarly in the liver, NOS1, NOS2 and COX1 expressions were not different between the heterozygous groups, and COX2 was undetectable. Protein profile: the protein biomarkers 14.27 kd, 21.08 kd, 5.8 kd and 14.15 kd were highly expressed ($p<0.01$) when the 2 groups with abnormal adult vascular function ($NOS3^{-/-KO}$ and $NOS3^{+pat/-mat}$) were combined and compared to the 2 groups with normal function (NOS3+mat/-pat and $NOS3^{+/+WT}$, $p=0.0001$).). The 7.7 kd and 82.8 kd protein biomarkers were repressed in the group with abnormal vascular function ($p=0.005$). A clear separation between offspring having abnormal vascular function versus those with normal was noted using PCA. **Conclusions:** The lower gene expression detected in $NOS3^{-/-KO}$ and $NOS3^{+pat/-mat}$ mice lead to the hypothesis that the altered vascular function seen in these mice as adults is due to fetal vascular programming effecting gene involved in the vascular development. In addition proteomics analysis also detected serum biomarkers that were differentially expressed by fetal programming. Hence abnormal adult vascular function induced by an unfavorable uterine environment may be traced to specific altered vascular gene expression and protein patterns.

P1-020

The Effect of Acute and Chronic Induced Hypoxia on BDNF Protein Expression in The Ovine Fetal Brain Near Term Hidekazu Nishigori, Delfina M. Mazzuca, Victor K. Han, Robert Gagnon, and Bryan S. Richardson. Departments of Obstetrics and Gynaecology, Physiology/Pharmacology, and Pediatrics, Children's Health Research Institute, University of Western Ontario, London Ontario N6A4V2Canada

Background: Brain derived neurotrophic factor (BDNF) plays an important role in the regulation of neuronal proliferation, differentiation, and connective plasticity during development and is dependant in part on active stimulus input to the brain. We therefore sought to determine the effect of acute hypoxia with umbilical cord occlusion (UCO) and chronic hypoxia with placental embolization (EMB) which are known to alter electrocortical activity and thereby stimulatory input within the brain, on BDNF levels in the near term ovine fetal brain. **Method**: Chronically instrumented fetal sheep were studied over two days near term; UCO group, n=7, complete cord occlusion for 2 min every hr over 6 hr each day; EMB group, n=7, latex microspheres injected into the common umbilical artery until arterial O_2 content (CaO_2) was reduced by 50%; and control group, n=6. Animals were then euthanized, the fetal brains rapidly extracted and fast frozen in liquid nitrogen, and then subsequently analyzed for BDNF using the Emax Immuno Assay system from Promega. **Results**: UCO caused brief severe 80-90% reductions in fetal CaO_2 ($p<0.001$) which returned to baseline levels by 5 min post UCO while EMB caused a sustained 50% reduction in fetal CaO_2 ($p<0.001$). In control group animals, BDNF levels measured 5.7 $\pm$ 0.2 (SEM) pg/mg in the cortex, which was significantly lower and with less variance, than that measured in the subcortex at 10.6 $\pm$ 1.4 pg/mg. UCO and EMB resulted in a marginal fall in BDNF levels in the cortex, to 5.0 $\pm$ 0.6 pg/mg (NS) and 4.8 $\pm$ 0.4 pg/mg ($p=0.08$), respectively, but with no change in the subcortex. **Conclusion**: We conclude that induced hypoxia decreases BDNF levels in the cerebral cortex of the ovine fetus near term, and more so with chronic hypoxia, which may relate to decreased stimulus input and contribute to altered neuronal connectivity over time.

P1-021

Reduction of Salt Sensitivity in Stroke-prone Spontaneously Hypertensive Rats Administered an AT_1 Receptor Antagonist During Suckling Lila Otani, Tomohide Yasumatsu, Megumi Murakami, and Tetsuo Murakami ; Division of Advanced Life Sciences, Graduate School of Agriculture, Kinki University 3327-204 Nakamachi, Nara, 631-8505, Japan

Background: Treatment of spontaneously hypertensive rats (SHR) during very early life with agents targeting the renin-angiotensin-aldosterone system (RAAS) leads to persistent reductions in blood pressure. In this study, antihypertensive therapy was started during suckling and the effect on blood pressure and salt sensitivity of stroke-prone spontaneously hypertensive rats (SHRSP) was determined. **Methods**: SHRSP, from 2 weeks to 4 weeks *postpartum*, were divided into a control group and an AT_1 receptor antagonist treated group (losartan; 100mg/L in drinking water). Both groups of animals were weaned at 4 weeks of age and then were fed a commercial diet with tap water *ad libitum*. Blood pressure was measured by the tail cuff method. Both the control and losartan treated rats were divided again at 10 weeks of age into a tap water group and a 1% saline solution group. Plasma aldosterone was measured at the 4 and

15 weeks of age. **Results**: The body weights of the losartan treated rats were similar to the control rats at the end of treatment. However, at 10 weeks of age, the body weights of the losartan treated rats were lower than the control rats. There were no differences in blood pressure between the control rats and the losartan treated rats. After salt loading from 10 weeks of age, blood pressure elevation in the losartan treated rats was moderate, compared to the control rats. The mean life span of the losartan treated rats (104 ± 78 days ; n=10) was significantly greater than the control rats (37 ± 17 days ; n=10). Plasma aldosterone concentrations of the losartan treated rats were lower than those of the control rats at 4 and 15 weeks of age. **Conclusion**: We have demonstrated that suppression of the renin angiotensin aldosterone system from 2 to 4 weeks of age reduced salt sensitivity. Thus, it is suggested that suckling is a crucial stage in the development of salt sensitivity in SHRSP.

P1-022

Programmed Coronary Dysfunction: Evidence for Attenuated Vasodilatory Prostanoid Production Robert D. Roghair, Fred S. Lamb, Thomas D. Scholz, Jeffrey L. Segar; Department of Pediatrics, Carver College of Medicine, University of Iowa, Iowa City, IA 52242 USA

Background: Antenatal dexamethasone (dex) exposure is a robust model for investigation of the fetal programming of postnatal cardiovascular dysfunction. Such dex exposure was utilized to discern the mechanisms responsible for programmed ovine coronary reactivity. **Methods**: Dex (0.28 mg/kg/d iv for 48 hours) was administered to pregnant ewes at 27-28 days gestation (term being 145 d). Coronary artery reactivity was assessed by wire myography at a postnatal age of 140 ± 7 days in dex-exposed and age-matched control lambs (N = 6). **Results**: Coronary rings from dex-exposed sheep exhibited enhanced vasoconstriction to angiotensin II and the thromboxane A_2 agonist U46619 ($P < 0.05$). Preincubation with 10^{-5} mol/L indomethacin increased the responsiveness of the coronary segments to both agents and mitigated the effect of dex exposure on coronary reactivity. Dex exposure was further associated with significant alterations in responsiveness to acetylcholine (increased vasoconstriction) and adenosine (decreased vasodilatation). Coronary artery expression of cyclooxygenase type-2 (COX-2) was restricted to the endothelium and subjectively decreased by antenatal dex-exposure. **Conclusion**: Early gestation dex exposure impairs cyclooxygenase (COX)-mediated vasodilatory prostanoid production and endothelial-dependent vasodilatation. Given the epidemiological association between selective COX-2 inhibitor therapy and coronary artery disease, inhibition of vasodilatory prostanoid production may contribute to programmed coronary artery dysfunction.

P1-023

Viability of IVF Calves Compared with AI Calves Born by Caesarean Section or by Vaginal Delivery Schmidt M, Walsoe L, Schregardus M, Avery B, Greve T; Veterinary Reproduction and Obstetrics Reproduction, The Royal Veterinary and Agric University, Dyrlaegevej 68, 1870 Frederiksberg, Denmark

Calves born after in vitro fertilisation (IVF) may have decreased neonatal viability. A number of IVF calves are delivered by caesarean section to overcome their potential weakness. However, this procedure may further compromise their viability . The present study compared neonatal parameters such as blood chemistry, organ weights, thermoregulation and raising behaviour in IVF calves with those of half siblings produced by artificial insemination (AI) , both groups born either by caesarean section or by vaginal delivery. Dairy heifers were pregnant either after receiving an IVF embryo fertilized with a dairy bull in TALP and cultured in SOF (n=14) or after artificial insemination (n=13) with the same bull. At D 278 of gestation, 8 IVF calves and 6 AI calves were delivered by caesarean section (CS) and 6 IVF calves and 7 AI calves were born vaginally (VAG). Instantly after birth, permanent catheters were inserted in jugular veins of the newborns and blood samples and body temperature were taken at 5 min, 2h, 1h, 12h, 2h, 22h,3h, 6h, 9h, 12h, 19h, 21h, 22½h, 24h. The calves were fed with 40 ml colostrum/kg at 2h, 6h, 12h + 21h and euthanized at 24h for collection and weighing of internal organs. Whole blood samples were analysed for pH, pCO_2, pO_2, hemoglobin, glucose, Na^+ and Cl^-. The data were analysed by Fishers Exact test and are given as LS means ± SEM values and a significance level of $P<0.05$. During the period of 24 h after birth, the caesarean delivered IVF calves compared with the AI calves showed lower mean blood pH (7.29∀0.02 vs. 7.34∀0.02), lowered Na^+ (140,5∀0,6 vs. 142,1 ∀ 0,7mM) lower relative weights of liver and colon, higher relative weights of heart and thymus, lower body temperature (38.11∀ 0.22 vs 38.52∀ 0.17 °C) and retarded raising behaviour. The tendency was the same for the vaginally born calves but differences were significant only for the relative weights of liver and thymus. The weight of thymus decreases with maturity and the heavier thymus was a sign of immaturity of the IVF calves. The lower blood pH, thermoregulation and delayed raising behaviour reflected a slower adaptation to postnatal life and the lower weight of the liver and colon may reflect a slower developmental growth of the gastrointestinal organ of those calves. The study documented that neonatal in vitro produced calves were more compromised during the first 24 h than their half siblings produced by artificial insemination when they were delivered by caesarean section. It can be concluded that IVF calves were more immature at birth than AI calves and that it was an advantage for those calves to be born vaginally.

Cancer

P1-024

High Birth Weight Increases Mammary Tumorigenesis in Rats Sonia de Assis and Leena Hilakivi-Clarke; *Department of Oncology, Georgetown University, 3970 Reservoir Rd. NW, Washington, DC 20057, USA*

Background. High birth weight, a proxy of elevated maternal hormone and growth factor levels, is associated with increased breast cancer risk in premenopausal women. To investigate the mechanisms involved in mediating the effects of high birth weight on the breast, we used an animal model of high birth weight, and determined its association with dam's pregnancy hormone levels, carcinogen-induced mammary tumorigenesis, and changes in mammary gland morphology and gene and protein expression. **Methods.** Pregnant female Sprague Dawley rats were fed diets containing either 16% (control diet) or 45% (obesity-inducing diet, OID) energy as fat during the extent of gestation. Blood was drawn on gestation day 17 for hormonal assays done using EIA kits. At birth, the dams and pups were switched to semipurified AIN93G laboratory diet. Mammary glands of offspring were obtained on postnatal day 21 (before puberty) and day 50 (mammary gland is most susceptible for malignant transformation) and mammary tumors were induced by administering 50-day-old rats 10 mg 7,12-dimethylbenz[a]anthracene (DMBA). **Results.** The OID increased pregnancy serum leptin levels ($p<0.04$), but had no effects on IGF-1 or estradiol levels. Offspring born to dams fed the OID had a significantly higher birth weight (mean±SEM: 7.1±0.19 g), compared to offspring of dams fed the control diet (6.2±0.1 g) ($p<0.001$). The mammary epithelial tree of the high birth weight offspring was denser ($p<0.035$) and contained more terminal end buds (TEBs); ie., structures that are susceptible for the initiation of breast cancer ($p=0.001$), compared to the controls. The number of proliferating cells was significantly elevated in the TEBs ($p<0.025$) of high birth weight rats. No alterations in apoptosis were noted. Consistent with the changes in mammary gland morphology, animals that had a high birth weight developed mammary tumors significantly earlier (tumor latency in high birth weight group: 11.7±0.82 weeks and in the controls: 14.6±0.87 weeks, $p<0.03$) and the tumors grew larger (tumor volume in mm^{-3} when first palpable and after 6 week follow-up; controls: 495±100 and 436±141; high birth weight: 466±94 and 2,283±666, $p<0.001$), compared to the controls. Mammary gland of high birth rats also expressed lower levels of ER-α ($p<0.015$), but higher levels of activated MAPK ($p<0.025$). **Conclusions.** In summary, high birth weight is associated with increased mammary tumorigenesis in rats, perhaps by programming the fetal mammary gland to exhibit higher epithelial density, more TEBs and increased cell proliferation within the TEBs. These changes are consistent with increased activation of MAPK, but it remains to be investigated how the reduced levels of ER-α in the mammary gland relate to mediating the effects of high birth weight on later increase in susceptibility to develop breast cancer.

P1-025

Maternal Hormone Levels and Perinatal Characteristics: Implications for Testicular Cancer Yawei Zhang, Barry I. Graubard, Matthew P. Longnecker, Frank Z. Stanczyk, Mark A. Klebanoff and Katherine A. McGlynn. Yale University School of Medicine, New Haven, CT; National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC; University of Southern California Keck School of Medicine, Los Angeles, CA; National Institute of Child Health and Human Development, NIH, DHHS, Rockville, MD; National Cancer Institute, NIH, DHHS, Rockville, MD, USA

Background: It has been hypothesized that testicular germ cell tumors (TGCT) originate *in utero*, perhaps due to an imbalanced hormonal milieu. Although it is difficult to test this hypothesis retrospectively, studies have examined associations between perinatal factors that may be related to hormonal exposures, and TGCT risk. It is unclear, however, how well these perinatal factors are correlated with hormonal exposures, particularly across different ethnic groups. **Methods:** To determine whether perinatal factors are good surrogate measures of hormone exposures for TGCT studies, the relationships between maternal levels of estradiol, estriol and testosterone and perinatal factors were examined among 300 participants of the Collaborative Perinatal Project (CPP). The CPP, conducted between 1959 and 1965 at 12 U.S. medical centers, was a prospective study of pregnant women and their offspring. For the current analysis, all mothers were pregnant with singleton male fetuses and were representative of populations at high (white Americans) or low (black Americans) risk of TGCT. Maternal hormone levels were determined in samples collected in both first and third trimesters. **Results:** Among white mothers, testosterone levels were negatively associated with height ($p<0.01$) and age ($p<0.01$), and positively associated with weight ($p=0.02$) and BMI ($p=0.01$). Estradiol levels were negatively associated with height ($p=0.03$). In contrast, among black mothers, estriol levels were negatively associated with weight ($p=0.03$) and BMI ($p=0.03$). Among white sons, birth weight was negatively associated with estradiol ($p=0.03$), while among black sons, birth weight ($p<0.01$), birth length ($p=0.04$), head circumference ($p=0.03$) and gestational age were associated with estriol levels. **Conclusions**: These findings, that perinatal associations with maternal hormone levels vary by ethnicity, indicate that perinatal characteristics should be used with caution as surrogate measures of hormone exposure outside a particular ethnic group. Among white populations, previous studies have reported increased risks of TGCT with lower maternal weight and increased age. As both variables were associated with decreased testosterone levels in the current study, lower maternal testosterone levels among white populations may be a risk factor for TGCT.

P1-026

Birth Length, Adult Height and Risk of Breast Cancer Paul R. Romundstad, Tom I. Lund Nilsen, Lars Vatten; *Department of Public Health, Norwegian University of Science and Technology, 7006 Trondheim, Norway*

Background: Studies have shown that birth size as well as adult height is positively associated with breast cancer risk, and also that birth size is associated with adult height. In this study we have investigated whether the relationship between birth size and breast cancer risk may be mediated by an increased body height in adulthood. **Methods:** 11,877 women born between 1920 and 1958 at St.Olavs hospital in Trondheim, Norway, with available birth and adult height measurement data, were eligible for the study. The women were followed from date of adult height measurement until the date of the first diagnosis of breast cancer, another cancer, emigration, death from other causes than cancer, or to the end of follow-up (31 December 2002), whichever occurred first. We used Cox regression with attained age as the time scale to investigate association between birth size and risk of breast cancer controlling for adult height and potential confounders. **Results:** Birth length was positively associated with breast cancer risk, relative risk (RR)= 1.23 (95% confidence interval (CI) 0.98-1.53) per standard deviation (2.2 cm) increase in birth length, after adjustment for birth year, length of gestation, ponderal index, birth order, maternal age, maternal marital status and socioeconomic status at childbearing. Women who were 53 cm or longer at birth had a relative risk of 1.7 (95% CI = 1.0-2.8) compared to women being 50 cm at birth. Adjustment for birth weight or ponderal index tended to strengthen the birth length association, while no association was observed between birth weight and breast cancer. When we restricted the analyses to pre-menopausal breast cancer the association increased to a RR of 1.42 (95% CI = 1.0-2.8) per standard deviation (2.2 cm) increase in birth length. After adjustment for adult height, the association between birth length and breast cancer risk was attenuated from a RR of 1.23 to a RR of 1.15 (95% CI 0.92-1.43) per standard deviation (2.2 cm) increase in birth length. The relative risk for women who were 53 cm or longer compared to women being 50 cm at birth, decreased from 1.7 to a RR of 1.4 (95% CI = 1.1-1.9). Further adjustment for the women's age at first birth and parity in a subset of the cohort did not materially change the results. **Conclusions:** The results provide evidence that the association between birth size and breast cancer risk may, in part, be mediated through an increased body height in adulthood, and that birth length may be of greater importance than birth weight.

P1-027

Breast Cancer After Prenatal Exposure to the Dutch Famine R.C. Painter*, S.R. de Rooij*, T.J. Roseboom*, P.M.M. Bossuyt*, C. Osmond#, D.J.P. Barker§, O.P. Bleker## ; * *Department of Clinical Epidemiology and Biostatistics, Academic Medical Center at the University of Amsterdam, Amsterdam, the Netherlands # MRC Epidemiology Resource Centre at the University of Southampton, Southampton, UK § Developmental Origins of Adult Disease Centre, University of Southampton # # Department of Obstetrics and Gynecology, Academic Medical Center at the University of Amsterdam, Amsterdam, the Netherlands*

Background High birth weight has been reported to be associated with subsequent breast cancer risk. The mechanisms underlying this association remain unclear. We investigated the effects of maternal undernutrition during gestation on breast cancer incidence in the offspring. **Methods** During a hospital visit, home visit or telephone interview we asked 475 women, aged 56 to 61 (average 58), born as term singletons around the time of the 1944-1945 Dutch famine, whether they had ever been diagnosed with breast cancer. There were 15 cases. We compared the cumulative incidence of breast cancer among those exposed in late (n=82), mid (n=77) or early (n=46) gestation to unexposed women (n=270). **Results** Nine women who were exposed to famine in utero had had breast cancer (hazard ratio 2.6, p=0.08). Women exposed to famine in early gestation reported a history of breast cancer almost 5 times more often than non-exposed women (8.7% v 1.9%; hazard ratio 4.8, 95% confidence interval 1.2 to 17.8). There was a non-significant increase in breast cancer history in those exposed in mid gestation (3.9% hazard ratio 2.1) and late gestation (3.7% hazard ratio 2.0). Adjusting for socio-economic status, body mass index and nulliparity did not attenuate the association between exposure in early gestation and breast cancer history up to the age of 61 (adjusted hazard ratio 7.8, 95% confidence interval 1.7 to 36.1). **Conclusion** Although numbers are small, this is the first evidence suggesting that fetal undernutrition followed by normal nutrition may lead to an increase in breast cancer incidence.

P1-028

Frailty Modeling of Colorectal Cancer Incidence in Norway: Indications that Individual Heterogeneity in Risk is Related to Birth Cohort Elisabeth Svensson[1], Tron A Moger[2], Steinar Tretli[1], Odd O Aalen[2], Tom Grotmol[1]; [1] The Cancer Registry of Norway, Montebello, 0310 Oslo, NORWAY; [2]Institute of Biostatistics, University of Oslo, NORWAY

Introduction: Some cancer types level off or decrease in incidence at older age groups, not following the Weibull hazard rate. This stagnation can be explained by the frailty model, which describes the population effect of mixing individuals who are susceptible, with high risk of cancer, with those that are non-susceptible, with almost non-existing risk of cancer even in the oldest age groups. Frailty, or susceptibility, is hypothesized as being established in utero or early in life, and the well-known hereditary colorectal cancer conditions comply with this definition. The novel perspective proposed here, is that a frailty phenomenon may also be present among ordinary sporadic cases of colorectal cancer, where exposure of exogenous factors acting early in life may determine future susceptibility.
Methods: Colorectal cancer incidence data for the Norwegian population aged 40 to 99 years, diagnosed between 1956 and 2000 were analyzed by a frailty model, where the frailty is compound Poisson distributed.
Results: The model provided an acceptable fit to the data. The estimated proportion of non-susceptibles dropped from about 95% to about 76% from the first cohort (1851-55) to the last cohort (1946-1950), in line with the rise in incidence of the disease during this period. According to the frailty modeling, the estimated number of genetic events necessary for a malignant lesion to develop in the colorectum, is 7-8, which accords with the present knowledge regarding colorectal carcinogenesis.
Discussion: The frailty phenomenon may be present in this cancer form and provides an intriguing way of looking at the disease, with only a subset of the population being susceptible for colorectal cancer.

P1-029

Birthweight, Growth Patterns, and Breast Cancer Risk Mary Beth Terry, Denise Esserman, Julie Flom, Tamarra James, Ying Wei; *Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY 10032 USA*

Background: The development and structure of mammary tissue, including epithelial and stromal tissue which define breast density, are influenced most at four critical time periods – intrauterine, puberty, pregnancy and lactation, and menopause. Studies suggest that markers of fetal growth are associated with adult health outcomes including inverse associations between birthweight and cardiovascular disease and positive associations with breast and other cancers. Few studies have included prospective measures of early childhood growth when examining these relationships. We were interested in whether birthweight was associated with age at menarche, adult body size, and mammographic density – indicators of future breast cancer risk – after accounting for potential mediation by childhood growth measures. **Methods:** We contacted former female participants of the New York site of the U.S. National Collaborative Perinatal Project (NCPP) who were born between 1959 and 1966 and prospectively followed for seven years (n=808). This study has received institutional review board approval. The NCPP collected prospective data on prenatal exposures, birthweight, placental weight, birth length and childhood growth measures at 4 months, 8 months, 1 year, 4 years and 7 years. We examined postnatal growth by 1) calculating rates of growth between each consecutive time period; and 2) categorizing subjects by major pattern of growth. We defined the following patterns of growth between birth and age 7: catch-up growth- women who increased weight percentiles by more than 15 percentile points; catch-down growth – women who decreased weight percentiles by more than 15 percentile points; and stable growth – women who remained within 15 percentiles of weight. We have collected adult health questionnaire data including detailed data on reproductive history and adult body size on 250 subjects. We used the questionnaire data to calculate age at menarche and adult body mass (BMI). Of these women, 75% have already undergone screening mammography. To date, we have collected 60% of these mammograms and have assessed mammographic density through semi-automated methods (Cumulus Software, Ontario Cancer Center). We examined the association between birthweight and postnatal growth patterns on age at menarche, adult body size, and mammographic density using quantile regression techniques. We also compared our results to standard least squares and logistic regression models. **Results:** Higher birthweight and high weight at age 7 was associated with an earlier age at menarche versus those with low birthweight and low weight at age 7 (12.1 versus 12.7, p= 0.07). Higher birthweight was independently associated with adult BMI at age 20. This association was stronger among the higher quantiles of adult BMI (beta (se): 4.4 (2.99); 3.08 (1.13); 1.91 (0.66); 1.3 (0.62), 1.6 (0.5) for the 95, 75, 50, 25, and 10th percentile, respectively). For comparison, the association was 2.44 (0.83) using least squares regression. Postnatal growth also had a strong independent association with BMI at 20 years, particularly for the upper quantiles of adult BMI. Women who experienced rapid catch-up growth were more likely to be overweight at age 20 (BMI $\geq$ 25) than women who experienced either stable or catch-down growth. Prematurity (< 37 weeks gestation) was also independently associated with higher adult BMI. Catch-down growth was associated with higher mammographic density but this association was mediated by adult body size. **Conclusion:** These results suggest the importance of both birthweight and postnatal growth patterns on indicators of breast cancer risk. They also suggest the importance of considering opposing influences of early life growth on breast cancer risk factors and the necessity of adjusting for the influence of growth patterns on adult body size when examining long-term effects on mammographic density. This study adds to the growing literature suggesting the important of early life events on shaping breast cancer risk.

P1-030

A Case-Control Study of Early Life and Growth Factors and Osteosarcoma Rebecca Troisi, Chester Douglass, Robert N. Hoover; Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD 20892-7246 USA and Harvard School of Dental Medicine, Boston, MA 02115 USA

Background The etiology of osteosarcoma is unclear, although prevailing hypotheses involve the role of growth and development. **Methods** Osteosarcoma patients under age 40 were recruited from 10 orthopedic surgery departments in the United States from 1994-2000. Controls (n=141) with other orthopedic conditions were frequency-matched to cases (n=158) on age, gender and the distance of their home from the

respective medical center. Information was collected by interview and from birth records. Unconditional logistic regression models including age and sex were used to estimate odds ratios (OR) and 95% confidence intervals (CI). **Results** Current height, age and sex-specific height percentiles, age at which current height was attained and at the start of puberty were not associated with osteosarcoma risk. However, there was some evidence of an earlier accelerated growth period in cases based on height comparisons with peers at various ages during adolescence. Specifically, cases appeared less likely to report being shorter at ages 9-10 and 12-13, and more likely to be taller at ages 15-16 compared with being about the same height as their peers. There was also a suggestion that earlier puberty (as represented by starting to shave and developing pubic hair) in males might be associated with reduced risk. Of several factors related to the subject's *in utero* exposure, including gestational age, birth order, birth length, mother's and father's smoking status during pregnancy and mother's alcohol intake and primary occupation, only high birth weight demonstrated a statistically significant association with osteosarcoma risk (OR = 3.9; CI = 1.7-10 for 4,000 g vs. 3,000-3,500 g). The data provided no evidence of elevated risk with prior bone trauma, as measured by history of fractures or extensive participation in sports, nor from radiation exposure from routine medical screening. **Conclusions** These data provide limited support for the hypothesis that osteosarcoma tumors are related to size at birth and early adolescence, whereas earlier puberty in males may be protective. The results for growth and development characteristics were not definitive, but suggest that these factors may be markers of true etiologic events to which they are weakly correlated. Given the sharpness of the young incidence peak, we feel the most likely timing of these events is during in-utero development, and believe that a more concerted effort to evaluate this aspect of etiology is warranted. Promising candidates include genes, nutrients, infectious agents and other variables that could play an important role in fetal bone development, and also may have independent effects on adolescent growth and development.

P1-031

Birth Size as Risk Factor for Endometrial Cancer Elisabete Weiderpass[1,2], Pal Romundstad[1,3], Lars Vatten[3]; The National Cancer Registry of Norway, Oslo, Norway 2. Department of Medical, *Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden, 3. Department of Public Health and General Practice, Norwegian University of Science and Technology, Trondheim, Norway.*

Background: Previous studies on the relation between birth size and the risk of endometrial cancer have shown inconsistent results, namely either a reduced risk by increasing birth weight or no association at all. **Methods**: The St. Olav Birth Cohort consists of birth records of 23,478 women born between 1920 and 1966 in Trondheim, Norway. Trained personnel abstracted information on birth length and birth weight. The follow-up period started from January 1961, or at age 20 if this occurred later. The follow-up was made through linkages with nationwide population based registries, and ended at the date of first diagnosis of endometrial cancer or another invasive cancer, emigration, death from other cause than cancer, or end of follow-up period (31 December 2002), whichever occurred first. We excluded from all analysis 128 with a cancer diagnosis before start of follow-up, 10 women due to missing information on birth length or birth weight, and 6 due to unknown date of migration, leaving 23,334 women eligible for analysis. **Results**: We confirmed 66 incident endometrial cancer cases occurring during follow-up. Neither birth length nor birth weight were associated with endometrial cancer risk in an age adjusted analysis (see table). Further adjustments for maternal or paternal occupation (if maternal occupation was not available), maternal height, and parity did not alter the results meaningfully.

Table. Women's birth size and relative risk (RR) for endometrial cancer

Birth size	# cases	PYR	RR[a] (95% CI)	p-trend[b]
Birth length (cm)				
<50	22	183,201	1.9 0.8-4.3	
50-51	36	287,034	2.3 1.1-5.0	
52+	8	198,913	1.0 reference	0.3
Birth weight (g)				
<3250	24	228,360	1.4 0.7-3.0	
3250-3749	31	269,257	1.6 0.8-3.3	
375+	11	171,531	1.0 reference	0.4

[a] Adjusted for age (categories;0-24,25-29,30-34,35-39,40-44,45-49, 50-54,55-70)
[b] p-value trend evaluated by scoring the birth size categories

Conclusions: In this relatively large prospective cohort study we found no clear association between birth weight and birth length and risk of endometrial cancer.

Clinical and Public Health Interventions

P1-032

The Contribution of African Leafy Vegetables in Alleviating Vitamin A Deficiency (VAD) in Buter-Mumias District, Kenya

Anne A. Musotsi, Kennedy M. Shiundu, Shadrack Oiye, Ruth K. Oniang'o; *Rural Outreach Program, Nairobi Kenya.*

Background

Vitamin A deficiency affects millions of children and pregnant women worldwide. Despite decades of progress in vitamin A supplementation, the micronutrient deficiency remains a huge crisis in many parts of the world. More than 50% of the world's population suffering from hypovitaminosis is found in developing countries. In Kenya, high prevalence and the negative consequences of the deficiencies resulting from inadequate intake of vitamin A have resulted in focused international efforts to alleviate them. Vitamin A supplementation and food fortification may be needed as short-to medium-term measures, but long-term improvements require dietary diversification with increased consumption of vitamin A-rich foods. This calls for a focus on concrete action programs as well as nutrition education.

Purpose of the study: The study was done to estimate the contribution of African Leafy Vegetables in the vitamin A intake of rural dwellers in Butere-Mumias district, Kenya. These vegetables form an important dietary source of beta carotene and have been found to have superior vitamin A content as compared to exotic vegetables, such as Kale and Cabbage. **Methods:** The study was a cross-sectional survey conducted in 2 divisions of the district: Butere and Khwisero. A sample size of 817 households from the 2 divisions was selected randomly. A structured questionnaire was used to collect demographic and socio-economic data. The Hellen Keller food frequency checklist was administered to all sampled households. 24-hour recall was done on a sub-sample of 141 households. 44 households prepared vegetables for ß carotene (precursor of vitamin A) analysis. Data was analyzed quantitatively with Statistical Package for Social Sciences (SPSS) Means, Standard deviation, Student's t-test and Pearson correlation coefficient were used. The beta carotene analysis was done using High Performance Liquid Chromatography (HPLC). **Results:** The average frequency of consumption of African leafy vegetables was 5.9 days for Khwisero and 4.6 days for Butere. African indigenous vegetables contributed up to 32.7 % (239.32µgRE) of total vitamin A intake. The predominant ALV was the cowpea leaves. Plant source foods were found to be predominant beta carotene source in the two divisions and had varying levels of ß carotene before and after cooking. The levels of ß carotene differed with former analyses. Household income does not significantly influence vitamin A source and is weakly and negatively associated with vitamin A intake. **Conclusions:** Despite the general trend of diminishing consumption of ALVs, they still form an important dietary source of vitamin A in Butere-Mumias district. Cowpea leaves are available throughout the year as they can withstand climatic stress. Preparation methods of ALVs may affect ß carotene levels. ALVs should be promoted and more research done to improve intake and preparation.

P1-033

Different Strokes for Different Folks: Supplementary Support in a Randomized Controlled Trial of Prenatal Care Suzanne C. Tough, Jodi E. Siever, David W. Johnston. Calgary Health Region, Faculty of Medicine, University of Calgary, Decision Support Research Team, Calgary, Alberta.

Background: Addition of supplementary support to physicians caring for prenatal patients, such as nurses and home visitors (HV), may improve use of community resources, support health and well-being, and allow physicians to concentrate on the medical needs of patients. **Purpose**: To examine characteristics of women who engaged in nurse support compared to those who engaged in home visitor support. To describe the relationship between the number of visits with a nurse/HV and the use of prenatal community-based resources. **Methods:** As part of a larger randomized control trial of prenatal care, 1155 women were eligible to receive no-cost supplementary nursing support and 577 were eligible to receive no-cost home visitor support. The nurse provided individualized prenatal nursing support, including emotional support and resource referral. The HV focused on social support, practical assistance, supporting optimal prenatal health and connecting the client/family with community resources. The client and the nurse/HV together determined the intensity and duration of the intervention based on pregnancy-related goals. Study participants completed 3 computer-assisted telephone interviews over the perinatal period (first trimester, 32-34 weeks gestation, and 8 weeks post delivery). Data collected included information on demographics, lifestyle, psychosocial health, network orientation, history of abuse, resource use, prenatal care, and satisfaction with services. **Results:** Women saw the nurse an average of 3.4 visits (range 0 to 11 visits), with 14.4% opting for no contact and 29.2% having 5 or more visits. Women with access to HV support averaged 1.6 visits (range 0 to 14), with 26.6% opting for no visits and 18.9% of women participating 3 or more times. Women more likely to have higher number of contacts with the nurse (4 or more visits) were characterized by first pregnancy (56.2% vs. 42.3%%; p<0.001) and good mental health (65.2% vs. 55.5%; p=0.027). Women who did not access the available Nurse were characterized by non-Caucasian ethnicity (18.4% vs. 13.0%; p=<0.001), low social support (18.0% vs. 12.6%), smoker (17.8% vs. 7.1%; p<0.001), and past history of food bank use (30.2% vs. 13.3%; p<0.001). Women more likely to have 3 or more visits with the HV were characterized by non-Caucasian ethnicity (24.5% vs. 16.9%; p=0.013), income less than $40,000 per year (29.4% vs. 14.6%; p<0.001), high distress (25.9% vs. 19.7%; p=0.010), and low social support (22.0% vs. 17.4%; p=0.023). Over 50% of all women used 3 or more community based resources, of which written material, prenatal classes and breastfeeding support were the most common. Women having a first birth, who smoked, were less than 25 years of age, and those with a history of abuse were more likely to use 3 or more resources over the perinatal period. Increased contact with either the Nurse or HV was associated with accessing more community-based resources (p<0.001). **Conclusions** Women who engaged in no-cost support with either a nurse or home visitor were more likely to access community-based pregnancy-related resources. The majority of women met their pregnancy-related goals within 4 visits with the nurse and/or 3 visits with the HV. Characteristics of women who preferred the nurse (first parity, good mental health) differed from those who preferred the home visitor (non-Caucasian, low social support, distressed) indicating that service delivery models should be developed in consideration of the population they intend to serve. Resource uptake is facilitated by supplementary support, which may ultimately reduce the burden on primary care providers to address non-medical, pregnancy related needs.

Data Analysis; Statistical Approaches

P1-034

Investigating "Reversal Paradox" Bias in a Study of Thinness at Birth and Adolescent Blood Pressure Darren Dahly, Linda Adair – University of North Carolina at Chapel Hill, Department of Nutrition, Carolina Population Center; Chapel Hill, NC 27516 USA

Background: A large body of research on the relationship between birth size and later blood pressure is often cited as the strongest evidence in humans for the Developmental Origins of Health and Disease (DOHaD) hypothesis. Typically, these papers report linear regressions coefficients as an estimate of the effect of birth size on adult blood pressure after controlling for adult body size. Although several DOHaD researchers have noted valid weaknesses in this method, including a potential bias due to reversal paradox, many researchers are still presenting their birth size/blood pressure work in this manner. Our goal was investigate the potential for reversal paradox bias in a study of thinness at birth and adolescent blood pressure. **Methods**: We used data from the Cebu Longitudinal Health and Nutrition Study (CLHNS), an ongoing community based study of 3080 infants born in Cebu, Philippines from 1983 to 1984. For this analysis, the sample (n=1074) was restricted to boys (~16 years old) with measures for current (1998) systolic blood pressure (SBP), body mass index (BMI) at birth, and current BMI. Using these data, we constructed the *early*, *combined*, *late*, and *interaction* models suggested by Lucas, Fewtrell, and Cole. We then investigated the possibility of a bias in these models due to reversal paradox. **Results**: *Early model*: Regressing SBP on birth BMI resulted in a coefficient representing a -0.231 mmHG (p=0.329) change in systolic blood pressure for a 1 unit increase in birth BMI. *Combined model*: Adding current BMI to the model increased the magnitude of the birth size coefficient by 52% to -0.439 mmHG (p=.051) per unit increase in birth BMI. These results are very similar to other effect estimates found in the literature. *Interaction model*: An added interaction term for birth and current BMI resulted in an estimated effect of birth BMI of 1.707 mmHG (p=0.336) and an estimated effect of current BMI of 2.790 (p=0.016), although the interaction term did not improve model fit. *Late model*: Including only current BMI in the model resulted in an effect estimate of 1.380 (p<0.000). We then considered bias due to a reversal paradox, which we might expect because current BMI is positively correlated with both birth BMI and SBP. To test for this effect we used a residual measure of current BMI adjusted for birth BMI in order to remove the correlation presumably causing the paradox. The estimated effect of birth BMI on SBP shifted towards the null considerably, to -0.211 mmHG per unit change in birth BMI (p=0.345), which is what we would expect if the model was indeed suffering from a reversal paradox. While the degree of reversal paradox bias can vary between studies, we are reasonably sure that the bias we observed is not an unusually large one. Studies which report adjusted and unadjusted birth size coefficients regularly report differences that would fall within the bias we detected. This bias could be large enough to have an impact on a substantial number of already published papers. **Conclusions**: Adjustment for current body size when investigating relationships between birth size and later blood pressure can result in a reversal paradox which may substantially bias effect estimates. This bias can be large enough to change fundamental conclusions drawn from the data and should always be tested for.

P1-035

Birth Weight, Catch-Up Growth and Current Body Weight in the Fetal Origins Hypothesis Mark S Gilthorpe (1); George TH Ellison (2), Yu-Kang Tu (1,3) (1) Biostatistics Unit, Centre for Epidemiology & Biostatistics, University of Leeds, Leeds, LS2 9LN, UK; (2) St George's, University of London, Cranmer Terrace, London SW17 0RE, UK; (3) Leeds Dental Institute, University of Leeds, Leeds LS2 9LU, UK.

Introduction: Some studies have questioned the validity of the fetal origins of adult disease hypothesis arguing that associations might be due, in part, to inappropriate statistical adjustment for variables on the causal pathway, creating the statistical artifact known as the 'reversal paradox'. A modified version of the fetal origins of adult disease hypothesis is that catch-up growth has a *stronger* impact on health in later life than birth weight. However, epidemiological evidence for this is often based on similar statistical models. Using vector geometry, we question how different/ equivalent the modified hypothesis might be when compared to the original hypothesis. Thus, we seek to identify, using vector geometry, the extent to which the reversal paradox might need to be considered when examining evidence for the modified hypothesis based on catch-up growth. **Methods:** Concepts of vector geometry are introduced to illustrate multiple regression analysis in relation to the modified fetal origins of adult disease hypothesis. Furthermore, we then explored the impact of adjusting variables by generating their z-scores and analyzing these in place of the original raw variables. **Results:** Geometrically it could be shown that the three covariates – birth weight, catch-up growth, and current body weight – only span 2-dimensional space. Regressing the outcome (adult disease) on any two covariates thus equates to projecting the outcome vector onto the plane spanned by the three covariate vectors. Consequently, the three possible regression models, where any two of the three covariates are considered, are equivalent and yield exactly the same model fit (R^2). Statistically, these models are simply re-parameterizations of the same model. If instead of modeling weight in kilograms, we use z-scores of body mass, the variance of birth weight is far smaller than that of current weight. Consequently, those with low birth-weight z-scores, and only slightly larger current-weight z-scores, might in fact gain less weight (in kilograms) than those with high birth-weight z-scores and only slightly lower current-weight z-scores. Thus, the relation between blood pressure and growth weight in kilograms might be different to that between blood pressure and growth weight expressed in terms of z-scores. **Conclusion:** Vector geometry is a useful tool for understanding multiple regression and the insights provided here illustrate that it is impossible to differentiate the effects of catch-up growth from that of current body weight on the adult disease outcome. Similarly, it is impossible to differentiate the effects of catch-up growth from that of birth weight. For these reasons, the impact of the reversal paradox on epidemiological evidence for the fetal origins of adult disease is the same for catch-up growth as it is for birthweight. Furthermore, when shifting to a z-score scale, it becomes an even greater challenge to interpret the impact of weight change upon the function of the human body.

P1-036

Lifecourse Functions: A Functional Data Approach to Lifecourse Analysis Robert M West Katie Harris, and Mark S Gilthorpe Biostatistics Unit, Centre for Epidemiology & Biostatistics, LIGHT, University of Leeds, Leeds, LS2 9LN, UK

Background: Functional data analysis has been successful in several applications (Ramsay and Silverman 2002) and has great appeal for lifecourse analysis. A fascinating example of the analysis of human growth data is given by Ramsay *et al.* (1995). For each individual, a longitudinal series of measurements is summarized by a function. Analyses are then carried out on these functional 'observations'. If sufficiently smooth functions are used, then derivatives can be calculated, thereby giving rise to the possibility of considering both the velocity and the acceleration of growth of an individual. Some of the available techniques are illustrated on the data from 'Study 1' provided on the DOHaD 2005 Congress website. In so doing the practicality of a functional approach is examined. Glucose measurements are taken in order to classify the participants of the study as diabetic (DM), glucose intolerant (GTT) or normal (OK) and that the value of this exercise is to identify subjects from their childhood measurements that have greatest or least risk of DM or GTT. **Methods:** There is a toolbox of functional methods available to those prepared to consider a functional analysis approach, including principal component analysis of functional data and several others. It is seen that it is not practical to analyse the full dataset as there are insufficient measurement occasions for some individuals for the functions to be reliably fitted. A further complication is that measurements are taken at different times. Once a function is fitted, however, that function can be sampled at regular intervals. Then the functional data techniques developed by Ramsay and others can be applied. These sampled values can then be used to select the aspects of the functions that vary most between individuals, thereby becoming features (i.e. childhood characteristics of interest) for classification. Plots of glucose measurements against childhood characteristics reveal no clear trends and therefore encourage use of a classification tree. **Results:** The functional approach is shown to have several merits, including useful graphical representations. Included in the childhood characteristics are minimum and maximum velocities of weight gain: note that weight gain can be negative: that is weight loss. These contribute significantly to the classification tree. **Conclusion:** There are challenges in applying a functional approach. When there are few measurements over the lifecourse, the fitting of functions does not appear reliable for all individuals. Such functional data is therefore discarded. On the other hand, when measurements are taken at many points, the functional form becomes a good representation of the data and functional data analysis is seen as an attractive option. Several new techniques become available since derivatives of the lifecourse functions, such as growth velocity and acceleration, are available. It is shown that graphical representations can yield valuable insights in the interpretation of the data. Finally the use of classification trees on childhood characteristics identified in this way proves to be of enormous value. **References:** Ramsey JO and Silverman BW (2002) Applied *functional data analysis: methods and case studies*, Springer. Ramsay JO, Bock RD, and Gasser T (1995) Comparison of height acceleration curves in the Fels, Zurich and Berkeley growth data. *Annals of Human Biology*, **22**, 413 426.

Endocrine and Metabolic Pathways

P1-037

Neonatal Sex Steroid Exposure of Female Rats Results in Insulin Resistance, Altered Size Distribution of Adipocytes and Sex Steroid Receptor Expression *Camilla Alexanderson[1], Britt-Mari Larsson[1], Malin Ottosson-Lönn[2], Agneta Holmäng[1]; [1]The Wallenberg Laboratory, Cardiovascular Institute, [2]Department of Internal Medicine, Göteborg University, Sweden*

Introduction Neonatal events might contribute to the development of disorders such as type 2 diabetes and obesity at adult age. Therefore, the aim of this study was to examine the effects of neonatal injection with testosterone (T) or estradiol (E) on insulin sensitivity, size distribution of adipocytes and adipose tissue gene expression. **Methods** Pups received one injection of T or E within 3 hours after birth. At 14 wks of age the rats were exposed to a euglycemic hyperinsulinemic clamp. Realtime-RT-PCR was assessed to study the mRNA expression of the sex steroid receptors. Adipocyte size was analysed with a computerized-image-analysis-system. **Results** Adult T-rats had an increased mesenteric adipose tissue weight, while adult E-rats had a decreased parametrial depot compared to controls. Experimental groups also showed alterations in serum sex steroid levels, decreased insulin sensitivity and altered adipocyte size distribution in the mesenteric fat depot only. Furthermore, sex steroid receptor mRNA expression was altered compared to controls. **Conclusions** Exposure of sex steroids, at an early age, contributes to reprogramming of adipose tissue with altered size distribution curve of the intra-abdominal fat depot. Besides this, sex steroid exposure

of newborn female rats results in insulin resistance with altered sex steroid receptor gene expression

P1-038

Upregulated Expression of PKCζ in Fetal Skeletal Muscle of Periconceptionally Undernourished Ewes AJ Buckley, MH Oliver, FH Bloomfield and JE Harding. Liggins Institute, Faculty of Medicine and Health Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand.

Background. Maternal nutrition has a strong influence on the *in utero* environment and can affect the development of metabolic processes in the offspring. There is substantial evidence, that in response to a prenatal nutritional insult, insulin sensitivity in the offspring is often increased in early postnatal life, but decreases rapidly with increasing age. Associated with this increased insulin sensitivity are upregulated expression levels of key insulin signalling proteins. We have previously shown that maternal undernutrition in the periconceptional period alters fetal glucose/insulin metabolism in late gestation, with increased insulin secretion in response to a glucose challenge, possibly reflecting accelerated maturation of fetal pancreatic function. However it is not yet known whether insulin sensitivity and the expression of insulin signalling proteins are also altered in the fetuses of periconceptionally undernourished ewes. **Aims.** To determine the effects of periconceptional undernutrition in sheep on the expression levels of key insulin signalling proteins in fetal skeletal muscle: insulin receptor β subunit, p85 subunit of PI3-kinase, and PKCζ. **Methods.** Ewes were either: well nourished (N) or undernourished from 61d before to 30d after mating (UN-60-30d). Well nourished ewes were fed to maintain body weight ±5%. Undernourished ewes were fed to achieve and maintain 15% weight loss after an initial two day fast. At 133d vastus lateralis muscle from singleton fetuses was obtained and rapidly frozen until analysis. Protein was extracted and the concentration quantified using the Bradford protein assay. Protein (20μg) was separated by SDS-PAGE and Western blot analysis was used to determine the expression levels of key insulin signalling proteins, namely the insulin receptor β subunit, the p85 subunit of PI3-kinase, and PKCζ, in the muscle samples. Protein expression in the muscle of the UN-60-30d fetuses is expressed as a percentage ± SEM of the normalized protein expression in N fetuses. **Results.** Expression of PKCζ was increased 2.5 fold in the skeletal muscle of the UN-60-30d fetuses when compared to the N fetuses. (250 ± 50 % vs 100 ± 20 %, p = 0.01). There were no differences between groups in the expression of skeletal muscle insulin receptor β subunit or the p85 subunit of PI3-kinase. **Conclusion.** Periconceptional undernutrition results in increased expression of a key insulin signalling protein in fetal muscle in late gestation, namely PKCζ. We speculate that this may reflect an adaptive response to reduced maternal nutrient supply around the time of conception, whereby the fetus increases insulin sensitivity and hence nutrient uptake for tissue growth in late gestation and after birth.

P1-039

Mismatching the Pre-and Postnatal Nutrient Environment Alters Endocrine Mechanisms of Cardiovascular Control in Sheep JK Cleal, KR Poore, JP Newman, P Englefield, R Henke, J Boullin, D Noakes*, MA Hanson, LR Green; Centre for Developmental Origins of Health and Disease, University of Southampton, UK. *Department of Veterinary Reproduction, Royal Veterinary College, UK.

Background: The renin-angiotensin system (RAS) and the hypothalamic pituitary adrenal (HPA) axis are candidate mechanisms linking altered nutrition in early life to cardiovascular dysfunction in adulthood. Previously we have shown that the blood pressure response to frusemide at 1.5 years in male sheep is reduced when the mismatch between pre- and postnatal nutrient environments is minimised (Cleal *et al.*, 2003). In this study we determined whether this effect persists into later life and investigated the underlying endocrine mechanisms. **Methods:** Welsh Mountain ewes (UK Animals Scientific Procedures Act 1986) received 100 % (C, *n*=37) or 50 % of total nutrient requirements (U, *n*=40) from 1-31 dGA, and 100 % thereafter. Offspring were fed *ad libitum* (CC, *n*=20 and UC, *n*=19) or at a level that reduced body weight to 85 % of individual target weight (predicted from 0-12 wk growth trajectory) from 12 to 25 weeks postnatal age and *ad libitum* thereafter (CU, *n*=17 and UU, *n*=21). Each group contained approximately equal numbers of males and females. At ~10 months of age carotid artery loops were created under general anaesthesia (3 % halothane/O_2). At both 1.5 and 2.5 years of age catheters were inserted into the carotid artery and jugular vein under general anaesthesia. RAS function was assessed at both ages using frusemide (5 mg/kg i.v. bolus), and at 2.5 years using captopril (500 μg/kg/h i.v. infusion), and angiotensin I (0.05 μg/kg i.v. bolus). Heart rate (HR) and mean arterial blood pressure (MAP) were monitored and plasma cortisol and ACTH were measured using an Immulite analyser. Data (mean ±S.E.M.) were expressed as area under the curve (AUC) and maximum response, and were analysed by ANOVA. **Results:** In males only at 1.5 years old, CU had a greater basal plasma ACTH concentration than CC (48.15 ± 6.50 *vs.* 30.15 ± 5.07 pg/ml, $P < 0.001$). In males only at 2.5 years old, CU compared to CC, had a greater basal cortisol concentration (1.90 ± 0.46 *vs.* 0.82 ± 0.29 μg/dl), a greater maximum MAP response to angiotensin I (42.91 ± 3.00 *vs.* 32.93 ± 2.46 mmHg increase) and a greater MAP AUC in response to frusemide (659.15 ± 85.65 *vs.* 411.10 ± 67.71) ($P < 0.05$). These effects were not seen if early gestation nutrient restriction was also received (UU). Following captopril infusion, there was no difference between nutrient groups in the MAP decrease in response to frusemide. **Discussion:** Postnatal nutrient restriction alters RAS function in adulthood, but not if early gestation nutrient restriction is also received. This is possibly influenced by changes in baseline HPA activity. The lack of differences in blood pressure effects following captopril administration indicates that the changes in RAS may be at a vascular level. Thus the RAS is affected by the mismatch between early gestation and postnatal nutrition. The effects are sex specific and could have consequences for renal/cardiovascular function in later life. Cleal JK *et al.*, 2003 Pediatric Research 53: 18A

P1-040

Prenatal Programming of Temperament Elysia Poggi Davis[1], Dawn A. Korsen[1], Laura M. Glynn[1], Chris Dunkel Schetter[2], Calvin Hobel[3], Aleksandra Chicz-Demet[1] & Curt A. Sandman[1]; [1]*Department of Psychiatry & Human Behavior, University of California, Irvine, 92868* [2]*Department of Psychology, University of California, Los Angeles,* [3]*Maternal Fetal Medicine, Cedars Sinai, Los Angeles, USA*

A significant proportion of variation in infant and adult health outcomes and disease risk is attributable to developmental processes during fetal life in response to a variety of social, psychological, physiological influences. Although a large and impressive body of literature supports this notion of fetal or developmental origins of health and disease, the major limitations in this field are that an overwhelming majority of these studies have (a) employed a retrospective design, and (b) used measures of birth phenotype (e.g. birth weight/size, length of gestation) as predictors of subsequent health outcomes. Thus, these studies are unable to ascertain the nature of the intrauterine milieu during fetal development, and it is unlikely that birth phenotype, *by itself*, plays a causal role in this relation. Intrauterine exposure to glucocorticoids (GCs) is one mechanism that may mediate these effects on the fetus. We examined the consequences of prenatal exposure to GCs for the development of behaviorally inhibited temperament, a risk factor for the development of social anxiety disorders. Although there is abundant evidence from animal models that increased prenatal GC exposure results in amplified behavioral inhibition and reduced coping in aversive situations later in life, there are few human studies examining the consequences of prenatal GC exposure. There is, however, evidence that prenatal maternal anxiety and prenatal exposure to synthetic GCs has consequences for fearful or behaviorally inhibited temperament. **Study 1:** Maternal salivary cortisol levels were measured longitudinally at four time points during pregnancy (14, 19, 25 and 33 weeks of gestation). Infant behavior was assessed at two months of age in 121 full term infants (58 male, 63 female) with subscales of the Infant Behavior Questionnaire (Gartstein & Rothbart, 2003). As expected, maternal cortisol levels increased from early to late pregnancy, $F(3,118) = 39.7$, $p < .05$, ? = .38. Interestingly, women with a steeper rise in cortisol had infants who displayed more fear behaviors at two months of age, after controlling for postnatal maternal psychological state (partial $r(121) = .22$, $p < .05$). **Study 2** Using the Children's Behavior Questionnaire (Ahadi, Rothbart, & Ye, 1993), we additionally assessed 28 of the children (12 male, 16 female), from the sample described above, when they were five years of age. Higher maternal cortisol during the early second trimester, but not later in pregnancy, was significantly related to fearful/behaviorally inhibited temperament (partial $r(27) = .52$, $p < .05$) after controlling for maternal psychological state at the time of child assessment. **Discussion** Elevated maternal cortisol during pregnancy has consequences for behavioral inhibition in infancy and childhood, with earlier exposures exerting a stronger influence. These data suggest that prenatal GC exposure has persisting consequences for development.

P1-041

Salivary Cortisol Responses to a Laboratory-based Psychosocial Stressor in Human Pregnancy and the Postpartum Period Ilona S. Fedcrenko, Aleksandra Chicz-DeMet, Alison L. Cammack, Pathik D. Wadhwa; *Department of Psychology and Social Behavior (ISF), Department of Psychiatry and Human Behavior (AC, ALC, PDW), University of California, Irvine; Irvine, CA 92697 USA.*

Background. In human pregnancy the reactivity of the hypothalamus-pituitary-adrenal (HPA) axis to acute pharmacological and physical stimulation is progressively attenuated with advancing gestation. Previous research suggests that this attenuation may persist into the postpartum period. However, very little is known about alterations of HPA axis responses to psychosocial stressors during pregnancy and in the postpartum period. **Methods.** We conducted a preliminary study in a sample of 40 pregnant women. Subjects were serially exposed to a standardized psychosocial laboratory stressor (i.e., the Trier Social Stress Test; TSST) at three time points in the first trimester of pregnancy at 16.28 ± 1.25 (SD) weeks gestation, in the third trimester at 30.94 ± 1.13 weeks gestation, and in the postpartum period at 14.98 ± 4.13 weeks after delivery. Saliva samples were collected immediately before and 1, 15, 30, 45, and 60 minutes after the TSST protocol for assessment of free cortisol. **Results.** Salivary concentrations of cortisol differed significantly across the three assessments ($F_{1.8,70.5}=56.01$, p<.001, ?=.59), with highest levels in the third trimester and lowest levels postpartum. On average, a significant salivary cortisol increase to the TSST was observed only at the postpartum assessment ($F_{2.5,97.4}=3.83$, p<.05, ?=.09). During pregnancy, salivary cortisol remained unaltered (1st trimester: $F_{2.3,91.4}=1.30$, n.s.) or decreased (3rd trimester: $F_{2.6,95.9}=3.65$, p<.05, ?=.09) after TSST exposure. The lack of cortisol response to the TSST during pregnancy cannot be explained by a lower overall number of subjects responding to the stressor. At each assessment, approximately one fourth of the participants (1st trimester and postpartum: 22.5%, 3rd trimester: 25.6%) showed an increase of more than 2.5 nmol/l relative to baseline (pre-stress) levels. **Discussion.** Consistent with previous research, an increase of cortisol levels was observed as pregnancy advances. Furthermore, in line with previous reports on blunted HPA axis responses to physical and pharmacological stimulation during pregnancy, cortisol levels did not increase in response to a psychosocial stress protocol during

pregnancy. A significant cortisol increase was observed in the postpartum period, however, this increase was smaller than increases previously reported for third-time exposures to the TSST. In summary, these data suggest an influence of pregnancy-driven changes in HPA axis responses to psychosocial stress that extend into the postpartum period.

P1-042

Intrauterine and Postnatal Growth Affects Serum Inhibin Levels in Male Infants

Fujii T, Itabashi K, Kitazawa S, Umeda Y; Department of Pedeatrics, Showa University Hospital, Tokyo; Child Medical Center, Showa University Northern yokohama Hospital, Yokohama

Recently it was suggested that small for gestational age(SGA) subjects have a tendency towards hypogonadism. This study investigated whether a difference in serum inhibin level in infancy was induced by various fantors influencing prenatal growth. The study population consisted of 29 boys and 24 girls less than 18 months old. Serum inhibin A and inhibin B were measured by two-site enzyme-linked immunosorbent assay (ELISA). To assess the independent association of serum inhibin A and inhibin B with all other evaluated variables, we used a backward stepwise multiple regression analysis. Independent variables were postnatal age, body weight SD score at blood sampling, gestational age, birth weight and its SD score. In male infants, birth weight SD score was associated with inhibin B concentration after adjustment for other factors (P=0.04). It was suggested that postnatal gonadal function was affected by intrauterine growth restriction in male.

P1-043

Socioeconomic and Racial Disparities in Insulin Resistance Among Adolescents: Results From a Community-Based Longitudinal Cohort Study *Elizabeth Goodman, MD, Stephen R. Daniels, MD, PhD, and Lawrence M. Dolan, MD, Heller School for Social Policy and Management, Brandeis University, Waltham, MA, and Divisions of Cardiology and Endocrinology, Children's Hospital Medical Center, Cincinnati, OH, USA.*

Background: Prior cross sectional research has shown that the inverse association between parent education (PE) and insulin resistance may a key factor underlying educational disparities in cardiovascular (CV) health. Obesity and type 2 diabetes, two important factors which increase risk for adult CV disease are associated with insulin resistance. Both are increasingly common in adolescents, especially among black adolescents and those from families with lower PE. **Objective:** To determine whether lower PE is associated with worsening insulin resistance in adolescents over a three year period and examine if there are racial disparities in this effect. **Methods**: Longitudinal cohort study of 1266 healthy non-Hispanic, black and white 5-12th graders in a suburban, Midwestern public school district. At baseline, a physical exam was done and a fasting morning blood sample was drawn for insulin, glucose, HDL cholesterol (HDL-C), and triglyceride (TRIG) measurement. BMI was calculated from measured height and weight. A parent provided information on PE. Three years later, BMI and fasting insulin and glucose were re-assessed. Insulin resistance was calculated using the homeostasis model (HOMA). BMI z scores were derived from CDC 2000 growth chart standards. Obesity was defined as BMI-for-age >= 95% or BMI >= 30. The influence of PE on HOMA at year 3 was examined using general linear models which adjusted for age, sex, race, HDL-C, TRIG, baseline HOMA, and baseline BMI z score. Analyses were then stratified by race and weight status to assess for effect modification. The influence of puberty and change in BMI z score were also assessed. **Results**: The cohort was 46% black, 51% female. 18.9% were obese. Highest level of PE ranged as follows: high school or less 22.5%, some college or vocational training after high school 28.8%, college graduate 28.0%, professional degree beyond 4-year college 20.7%. There were no sex differences in PE, but black youth came from families with lower PE (p<0.001). Blacks and those with lower PE had higher BMI z score and increased HOMA at baseline and follow up (p<0.001 for all). In multivariable models, lower PE was significantly associated with worsening insulin resistance (β = 0.50, SE=0.12). Neither puberty nor change in BMI z score added significantly to the model. No interaction was noted between PE and race. However, PE's effect was more than 4-fold stronger among obese youth (β = 1.50, SE=0.51) compared to those who were not obese (β = 0.32, SE=0.09) and race became non-significant in analyses stratified by weight status. Estimated marginal means from multivariable models are shown below:

Estimated Marginal Means for Follow up HOMA model of insulin resistance*

		Total		Non-obese			Obese		
Parent Education	Mean	SE	p	Mean	SE	p	Mean	SE	p
High school or less	5.04	.26	0.001	3.73	.21	0.003	10.43	.96	0.003
Some college vocational training	4.21	.23		3.49	.19		6.92	.75	
College graduate or higher	3.41	.23		2.87	.18		5.44	1.00	
Professional degree	3.72	.23		2.89	.21		6.72	1.39	
Race			0.014			0.258			0.242
Black	4.43	.19		3.26	.16		8.01	.67	
White	3.76	.17		3.12	.13		6.75	.83	

*adjusted for age, sex, HDL-C, TRIG, baseline HOMA, and baseline BMI z score.

Conclusions: Lower PE is related to increased insulin resistance and worsening insulin resistance over time among adolescents. There are no racial disparities in this effect. However, obesity is an important effect modifier; the effect of PE is especially marked in obese youth.

P1-044

Metabolic Response to Feed Restriction and Re-feeding as Affected by Late Gestation Nutrition and Selection Line in Lambs Husted S.M.[1], Nielsen M.O.[1] & Ingvartsen K.L.[2]; [1]Dept. of Animal & Veterinary Basic Sciences, The Royal Veterinary & Agricultural University, Grønnegårdsvej 7, DK-1870 Frederiksberg C, Denmark [2] Dept. Animal Health; Welfare & Nutrition, Danish Institute of Agricultural Sciences, P.O. Box 50, DK-8830 Tjele, Denmark.

Background Substantial evidence now suggests that prenatal nutritional restriction during sensitive periods results in life-lasting so-called metabolic programming. After birth, metabolic adaptability to variations in nutrient supply will be altered with potential negative impact on animal productivity. We aimed to investigate whether metabolically programmed lambs exhibit different metabolic responses to a feed restriction period followed by re-feeding compared to non-programmed lambs. And whether genetic selection for growth rate versus meat content in the carcass, affects the manifestation of metabolic programming and adaptability. **Materials and methods** 40 Shropshire lambs, born to 20 twin-pregnant ewes, were used. 2 ram selection lines were used: high daily weight gain (GROWTH) versus high slaughter quality (MEAT), and 2 different feeding levels were offered to the pregnant dams during the last 6 weeks pre-partum: according to Danish requirements for energy and protein (NORM) or 60% of norm (LOW). Birth weights of NORM lambs (4.62±.13 kg) were significantly higher than LOW lambs (3.69±.11 kg, P<0.0001). Post-partum all dams and lambs were fed the NORM diet *ad libitum*. At 20 weeks of age, lambs were subjected to a 7 day feed restriction period (50% of *ad libitum* net energy intake), followed by re-feeding. Plasma samples were obtained at 11am at day –2 and –1 (fed state), 2, 4, 6, 7 (feed restricted state), and at 1, 3, 5, 7, 48 and 120 hours (re-fed state), and analysed for glucose, non-esterified fatty acid (NEFA), ß-hydroxybutyrate (BOHB), urea and insulin. Data were analysed as repeated measurements by the mixed model procedure in SAS. All values are given as LSMEANS±SEM. **Results** We could not establish any clear significant effects of neither pre-natal nutritional management nor selection line on plasma metabolite or insulin concentrations before or during the feed restriction period. Never the less, the LOW group tended to be more efficient at maintaining glucose homeostasis during feed restriction compared to NORM lambs, and NEFA concentrations were found to increase more and stay higher in LOW-GROWTH compared to the other 3 groups. Treatment differences were observed in response to re-feeding: LOW lambs had faster increases in glucose concentrations than NORM lambs and continued to have higher glucose levels 48 hours after re-feeding (4.01±0.11mM vs. 3.70±0.11mM; p=0.043). Insulin concentrations acutely increased in both NORM and LOW in response to re-feeding, then dropped and stabilized in NORM, but continued to increase, and was significantly higher in LOW than NORM 120 hours after re-feeding (0.77±0.07 mM vs. 0.38±0.06 mM, p<0.0001). **Conclusion** The results agree with our earlier findings, where 20 wk old LOW lambs maintained higher glucose concentrations and released more NEFA during a 24 hour fast than NORM lambs (Husted et al., 2004). Improved ability to maintain glucose homeostasis in LOW lambs agrees with the general perception of lowered insulin sensitivity in peripheral tissues and lowered pancreatic sensitivity to glucose in metabolically programmed individuals. The larger releases of NEFA during feed restriction and of insulin during re-feeding, however, do not. More severe feed restriction challenges than in this study are required to reveal the tissue specific metabolic adaptations induced by metabolic programming and interactions with genetic properties. **Reference** Husted S., Thygesen M.P. and Nielsen M.O. (2004) Journal of Animal and Feed Sciences, 13, Suppl. 1, pp: 491-494

P1-045

Maternal Taurine Supplementation in the Late Pregnant Rat Stimulates Postnatal Growth and Induces Abdominal Obesity and Insulin Resistance in Adult Offspring Karin Hultman[1,2], Camilla Alexandersson[1], Mats Sandberg[3], Agneta Holmäng[1], Thomas Jansson[3,4]; [1]Wallenberg laboratory, [1]Cardiovascular Institute, [2]Institute of Clinical Neurosciences, [3]Institute of Physiology and Pharmacology, The Sahlgrenska Academy, Göteborg University, Sweden and [4]Dept of OB/GYN, University of Cincinnati, USA

Background: The mechanisms linking restricted fetal growth to the development of type-2 diabetes, obesity and cardiovascular disease in adult age remain to be fully established. This association appears to be particularly strong when intrauterine growth restriction (IUGR) is followed by a rapid postnatal catch-up growth. Taurine is an essential amino acid during fetal life and an adequate taurine supply is critical for normal beta-cell development and important for insulin action. IUGR is associated with decreased fetal plasma concentrations of taurine. A reduced supply of taurine may represent one mechanism by which glucose metabolism is programmed in utero. **Aim:** The aim of this study was to examine whether maternal taurine supplementation in late pregnancy affects postnatal growth, adult body composition and insulin sensitivity in IUGR and normal offspring. **Methods:** Bilateral uterine artery ligation or sham-operation was performed on day 18 of gestation (term = 23 days of gestation) in pregnant rats. Taurine (2 % w/v) was added to the drinking water during the last five days of pregnancy to half of the animals. In separate experiments fetal and maternal plasma taurine concentrations were determined using HPLC. Postnatal growth in sham (n=22), sham/taurine (n=22), IUGR (n=22) and IUGR/taurine (n=24) offspring was recorded and animals were further studied at 3 months of age. Peripheral insulin sensitivity was determined by a euglycemic hyperinsulinemic clamp and endogenous insulin production was assessed using a hyperglycemic clamp. Plasma FFA was determined using an enzymatic colorimetric method. Parametrial/epididymal and retroperitoneal fat depots were weighed. **Results:** Taurine supplementation resulted in

a marked elevation of maternal taurine concentrations and a 2-fold increase of fetal plasma taurine concentrations. In offspring with normal birth weight, prenatal taurine supplementation markedly stimulated postnatal growth. Furthermore, in sham/taurine females abdominal fat depots and plasma free fatty acids concentrations were increased and peripheral insulin sensitivity was reduced. Insulin sensitivity was unaltered in IUGR and IUGR/taurine offspring. However, whereas IUGR offspring showed almost no catch-up growth, 50% of IUGR/taurine animals had reached the body weights of the controls at 3 months of age. Animals in this subgroup had increased abdominal fat depots and reduced insulin sensitivity compared to non-catch up IUGR/taurine rats. **Conclusions**: These data suggest that fetal taurine supply is an important determinant for postnatal growth, accumulation of abdominal fat stores and insulin sensitivity. Furthermore, an increased postnatal growth rate is associated with adult abdominal obesity and insulin resistance in both IUGR animals and in offspring with normal birth weight.

P1-046

Cord Plasma from Large Newborns Stimulates Matrix Metalloproteinase Activity in Cultured Endothelial Cells Erlingur Johannsson, Tore Henriksen and Per Ole Iversen; *Center for Sport and Health Sciences, Iceland University of Education, Laugarvatn, Iceland; Department of Nutrition, Institute of Basic Medical Sciences and Department of Gynecology and Obstetrics, Rikshospitalet, University of Oslo, Norway*

Background/aim A growing body of evidence indicates that inadequate nutritional exposure *in utero* is related to increased risk of future chronic disease. In addition to perinatal complications, large babies (birth weight > 4 kg) appear to be prone to later cardiovascular diseases. The causal link beween fetal nutritional conditions and later risk of disease is, however, unknown. The vascular endothelium is generally a target site in the pathogenesis of cardiovascular diseases, and factors in the circulation may already at the fetal stage induce properties in the endothelial cells that later may predispose to disease. Matrix metalloproteinase (MMP) activity is an indicator of endothelial activation and a modulator of extracellular matrix. We therefore examined the effect of plasma from cord blood of infants with different birth weights on MMP activity in cultures of endothelial cells. **Materials and methods** We sampled venous cord plasma of otherwise healthy mothers with uncomplicated pregnancies and who delivered with planned caesarean sections after the 37th gestational week. Primary human umbilical cord endothelial cells (HUVECs) of indifferent donors were exposed to plasma samples (20% v/v) for 48 h. The harvested supernatant was then subjected to zymography in order to determine MMP-activity (Thorud et al., *Am J Physiol* 289: R389-R395, 2005). Cord blood lipids and insulin were also determined. **Results** The ratio between MMP activity in supernatant of cells exposed to samples from large newborns (n = 4) relative to newborns with birth weight of about 3 kg (n = 4), was 4.1, indicating a substantial endothelial activation in the former group. The gel activity pattern suggested activation of primarily the MMP-9 subtype. The table below reveals no differences (P > 0.05) in cord blood lipids or insulin between newborns with birth weights (b.wt.) in the highest quartile (Qhigh) compared to those in the lowest quartile (Qlow):

B.wt./parameter	Triglyceride (mM)	HDL (mM)	LDL (mM)	Insulin (pM)
Qlow, b.wt. < 3.1 kg	0.13 ± 0.10	0.82 ± 0.21	0.71 ± 0.32	29.8 ± 26.9
Qhigh, b.wt. > 4.0 kg	0.26 ± 0.16	0.65 ± 0.12	0.51 ± 0.07	49.6 ± 20.8

Values are the means ± SD, n= 7-8.

Conclusion Cord plasma from large newborns activates MMP in vascular endothelium as compared to plasma from near-normal weight infants. The cord blood lipids or insulin did apparently not affect MMP activity. The nature of the compounds responsible for the endothelial activation, remains to be identified.

P1-047

Impact of Dietary Protein Restriction on One Carbon Metabolism in the Rat Satish C. Kalhan, Prabhu S. Parimi, and Richard W. Hanson; *Schwartz Center for Metabolism & Nutrition, Case Western Reserve University at MetroHealth Medical Center, Cleveland, OH 44106 USA*

Dietary protein restriction during pregnancy in the rat has been used extensively to examine fetal metabolic imprinting. It has been shown to cause hypermethylation of the genomic DNA in the fetus and hypertension in adult life. The mechanism of these changes remains unknown. Since methionine metabolism is linked to several methylation reactions, we hypothesized that dietary protein restriction will have significant impact on transmethylation and transsulfuration of methionine. In the present study we have examined the impact of dietary protein restriction on changes in methionine metabolism in non-pregnant rats. Animals were placed on casein-based 6% (low protein, LP) or 24% (normal protein, NP) protein diet for 5-7 days. They were allowed to feed ad lib and housed in metabolic cages. At the end of the dietary period, tracer isotope infusions were given to quantify methionine kinetics, the plasma and tissue levels of amino acids and the activity of key enzymes involved in methionine metabolism were measured. An LP diet caused a significant increase in plasma serine (526 ± 130 vs. 225 ± 75 μmole.L^{-1}, mean ± SD), glycine (569 ± 172 vs. 292 ± 88 μmole.L^{-1}), citrulline and ornithine levels as compared with NP diet. There was no change in plasma cysteine, homocysteine and glutathione levels. LP diet caused an increase in hepatic free glycine and serine levels, and a decrease in free taurine levels. Hepatic S-adenosyl homocysteine (SAH) levels were significantly lower in the LP group (LP: 15.7 ± 50, NP: 24.4 ± 5.6 nmole.g^{-1} wet weight) as compared to NP animals, so that the S-adenosyl methionine (SAM) /SAH ratio was significantly higher in the LP group (5.2 vs. 3.5). The LP diet decreased the activity of hepatic cystathionine ß synthase (LP: 0.31 ± 0.04, NP: 0.46 ± 0.17; p<0.02) and cystathionine ? lyase (LP: 0.82 ± 0.27, NP: 1.07 ± 0.09; p<0.01), which was associated with a decrease in the rate of transsulfuration measured by tracer isotope. The exact mechanism responsible for these changes is not known, however changes in methionine metabolism could impact the methylation of key regulatory genes resulting in programming and imprinting.

P1-048

Modulation of the Ontogenic Adrenal Steroidodgenic Capacity in the Fetal Sheep by Maternal Betamethasone Administration in Late Gestation Shaofu Li[1], John RG Challis[3], Timothy JM Moss[1, 2], John P Newnham[1, 2], Deborah M Sloboda[1, 2]
[1]*School of Women and Infants Health, The University of Western Australia, Perth, WA* [2]*Women's and Infants' Research Foundation, Subiaco, WA;* [3]*Depts of Physiology and Obstetrics and Gynecology, the University of Toronto, Canada*

Background: We have previously observed an evolving spectrum of hyper- and hypo-pituitary-adrenal responsiveness in sheep over the course of prenatal and postnatal life in response to prenatal betamethasone. The aim of the current study was to determine mechanisms underpinning these effects in the fetal and newborn sheep adrenal. **Methods:** Pregnant ewes carrying male fetuses were injected with 1 (104 days of gestation; dG), 2 (104, 111 dG) or 3 (104, 111, 118 dG) doses of betamethasone (0.5mg/kg body weight) or saline. Maternal and fetal plasma was collected prior to (84), during (109, 116), and after betamethasone at 132-133 and in offspring at 6 weeks of age. Plasma adrenocorticotropin (ACTH) and cortisol (F) were measured using radioimmunoassay. Fetal pituitaries and adrenals were collected and adrenals processed for mRNA determination of steroidogenic enzyme P450c17, ACTH receptor (ACTH-R), glucocorticoid receptor and 11ßHSD2 using real time RT-PCR. **Results:** One dose of maternal betamethasone reduced fetal pituitary weight at 109 dG (P=0.027) and three doses decreased pituitary and adrenal weights at 133 dG (P=0.052; P=0.01). Three doses of betamethasone resulted in a modest increase in adrenal weight at 6 weeks of age (P=0.08). Fetal and newborn cortisol levels were unaltered by maternal betamethasone administration. ACTH levels were reduced at 6 weeks of age after 3 doses of betamethasone (P=0.038). Adrenal P450c17 and 11ßHSD2 mRNA levels increased with increasing prenatal age in both groups (P<0.05). Adrenal P450c17, ACTH-R, 11ßHSD2 mRNA levels were higher at 6 weeks of age compared to prenatal time-points in both groups. Adrenal P450c17 mRNA levels were reduced at 109 dG after one dose of betamethasone (P=0.004), and modestly reduced after two (at 116 dG; P=0.1) or three doses (at 132 dG; P=0.057), but not in newborn lambs at 6 weeks of age. There was no effect of maternal betamethasone administration on mRNA expression levels of all other adrenal factors measured. The ratio of F to P450c17 mRNA increased with increasing pre- and postnatal age in both groups but was higher after one dose (at 109 dG; P=0.004), two doses (at 116 dG; P=0.03) or three doses (at 132 dG; P=0.029) of betamethasone, but not in offspring at 6 weeks of age. **Conclusions:** Fetal exposure to maternal betamethasone decreases fetal adrenal P450c17 enzyme mRNA expression late in gestation, but does not alter fetal or newborn adrenal ACTH-R, glucocorticoid receptor or 11ßHSD2 mRNA levels in sheep. Since P450c17 is a rate-limiting enzyme in the production of cortisol, the observed increase in fetal cortisol to P450c17 ratio may be indicative of an increase in fetal adrenal efficiency in producing cortisol. Alternatively, a reduction in fetal cortisol clearance may have occurred. It appears that the fetus has the capacity to adapt in a way that maintains normal cortisol levels despite significant decreases in P450c17 mRNA levels, although this does not persist to 6 weeks of postnatal age. Our observations support the concept that the HPA axis is a dynamic and robust endocrine system that permits the fetus to adapt to a changing environment.

P1-049

Enhanced Insulin Sensitivity in Fetal Sheep with Placental Insufficiency and Intrauterine Growth Restriction (IUGR) Sean W. Limesand[1,2], Danielle Smith[1], Paul J. Rozance[1], William W. Hay, Jr.[1]; *[1] Perinatal Research Center, Department of Pediatrics, University of Colorado Health Sciences Center, Aurora CO; [2] Department of Animal Sciences, University of Arizona, Tucson AZ*

Background Numerous human epidemiological studies have shown that prenatal nutritional insufficiency leading to IUGR is associated with later life metabolic disorders, such as insulin resistance, glucose intolerance, and overt type 2 diabetes mellitus. Insulin secretion increases in response to nutrient concentration changes during the second half of gestation; however, this response is reduced in fetal sheep with IUGR due to placental insufficiency, resulting in low circulating concentrations of insulin in the fetus. The aim of this study was to examine glucose metabolism in fetal sheep with placental insufficiency and IUGR under basal conditions and during a glucose clamp to determine their capacity to metabolize glucose at low plasma insulin concentrations. **Methods** Fetal glucose metabolism was determined in IUGR fetuses, generated by exposing pregnant ewes to elevated ambient temperatures during the middle third of gestation, and pair-fed control fetuses near term at basal and hyperglycemic states. The Fick principle was used to calculate umbilical glucose uptake and, in conjunction with D-[^{14}C(U)]glucose, rates of fetal glucose utilization and oxidation. Glucose transporter 1 (Glut1) levels were determined by immunoblot in liver and brain tissues; Glut 1 & 4 were analyzed in skeletal muscle. **Results** Fetal results are presented in Table 1 with asterisks distinguishing significant differences between treatment groups at steady states. Relative amounts of Glut1 per total protein were not different between control and IUGR fetuses for liver or skeletal muscle, but

Glut1 was greater in the brain of the IUGR fetus. Glut4 levels also were not different between treatments in fetal skeletal muscle. **Conclusion** Plasma glucose clearance rate for the IUGR fetuses was not different from the control fetuses even though plasma insulin concentrations were significantly lower. Predicted glucose utilization rates (Hay, etal., 1988) at the studied glucose and insulin concentrations indicated that fetal glucose utilization rate in the IUGR fetuses was 54% of control at basal and 81% at hyperglycemia. Therefore, fetal tissues had adapted to their hypoglycemic environment by developing mechanisms to promote glucose utilization, possibly via enhanced insulin action.

Table 1	Basal Study Period		Hyperglycemic Study Period	
	Control	IUGR	Control	IUGR
Plasma Glucose (mmol/L)	1.05±0.06	0.63±0.09*	2.31±0.09	2.37±0.09
Plasma Insulin (ng/mL)	0.26±0.04	0.09±0.01*	0.60±0.10	0.17±0.04*
Blood Flow (ml/min/kg)	164.0±7.3	126.0±22.6*	152.3±8.3	119.8±5.7*
Umbilical Glucose Uptake (μmol/min/kg)	25.0±1.4	16.9±4.1*	53.0±2.6	57.9±6.9
Glucose Utilization Rate (μmol/min/kg)	26.5±2.4	28.1±6.0	53.9±2.8	53.3±13.8
Glucose Production (μmol/min/kg)	1.5±1.6	11.2±1.8*	0.9±1.9	-4.6±6.7
Glucose Oxidation Rate (μmol/min/kg)	16.6±1.0	10.8±1.5*	26.4±1.6	23.7±1.0

P1-050

Angiotensin I Converting Enzyme (ACE) and Angiotensin II Receptor 1 (AT_1) Inhibition During Pregnancy and Lactation: Effect on the Renin-angiotensin System (RAS) of Adult Offspring Karen Lucasechi Lopes, Luzia N. S. Furukawa, Joel C. Heimann; Nephrology Section of the University of São Paulo School of Medicine, São Paulo – Brazil

Objective: To evaluate the effect of RAS inhibition during the perinatal period on adult offspring. **Methods**: Female Wistar rats were fed a normal (NSD 1.3% NaCl) or low (LSD: 0.15% NaCl) salt diet since 8 weeks of age. Body weight (BW) was evaluated every week from weaning. At 12 weeks of age, they were matched with adult males. The dams were divided in 3 groups: captopril 100mg/L (CAP), losartan 200mg/L (LOS), and control (CON). These drugs were diluted in the drinking water during pregnancy and lactation. After birth, only 8 pups (4 males - M and 4 females - F) were kept with their mothers. BW was measured at birth and every week since weaning. Protein expression (Western blotting) of the RAS components, ACE (fluorimetric method) and plasma renin activities (PRA) determinations were performed in adult offspring. **Results** (mean±SEM): BW (g) was lower ($p<0.001$) at the birth in NSD M and F offspring of the CAP (M- 6.1±0.06 - n=40, F- 5.8±0.08 - n=34) and LOS (M- 6.3±0.11 - n=53, F- 5.9±0.10 – n=50) groups compared to CON (M- 6.5±0.08 – n=50, F- 6.2±0.09 – n=40). PRA (ng/mL.hr^{-1}) was lower ($p<0.01$) in adult LSD+CAP (3.9±1.1 - n=5) than in LSD CON (8.2±0.9 – n=11) males. Similar results were observed in females (LSD+CAP 2.9±0.9 – n=8, LSD CON 6.9±1.2 – n=5). ACE activity (nmol His-Leu/min/mg) was lower ($p<0.05$) in female LSD+CAP (90.8±7.7 – n=6) than in LSD CON (116.3±7.2 – n=10). The AT_2 protein expression (arbitrary units) in kidneys from male offspring were higher ($p<0.05$) in NSD+LOS (13242±2078 – n= 3) and NSD+CAP (11487±2551 – n= 3) compared to NSD CON (3961±386 – n=2). **Conclusions**: ACE and AT_1 blockade during pregnancy and lactation induces alterations in the RAS from adult offspring.
Supported by FAPESP.

P1-051

IGF-II and IGFBP-2 are Associated with Components of the Metabolic Syndrome: Cross-sectional Data from the 65-year Follow-up of the Boyd Orr Cohort Richard M Martin[1], Jeff Holly[2], Elise Whitley [1], Andrew Nicolaides[3 4], George Davey Smith[1], Maura Griffin[4], Niki Georgiou[3], David Gunnell[1] *([1]Department of Social Medicine & [2]Department of Surgery, University of Bristol, [3]Cyprus Institute of Neurology and Genetics, [4]Vascular Noninvasive Screening and Diagnostic Centre, London)*

Background: Recent prospective epidemiological studies suggest that higher insulin-like growth factor-I (IGF-I) levels are associated with lower risks of type 2 diabetes and coronary heart disease (CHD). However, associations of IGF-II and the IGF-binding proteins (IGFBPs) have received little epidemiological attention. We investigated the cross-sectional relationship of the IGF-system in adulthood with the presence of coronary heart disease, diabetes, ultrasound measures of atherosclerosis and components of the metabolic syndrome. **Design:** Cross-sectional analysis of data from 799 participants (728 with blood samples; 339 with carotid ultrasound scans) in the Boyd Orr cohort who attended for follow-up examinations and venepuncture in 2002-04 when aged 63-84 years. **Outcomes**: Regression coefficients express the change in outcome associated with a standard deviation change in IGF. Fully adjusted models control for age, sex, social class in childhood, household food expenditure and birth order, social class in adulthood, alcohol consumption, smoking status, exercise and height. Skewed data were log transformed. **Results:** IGF-I was positively associated with log HOMA, a measure of insulin resistance (coefficient: 0.10; 95% CI: 0.03, 0.18) and with blood pressure (1.56 mmHg; 0.40, 2.72) but there was little evidence that levels of IGF-I were associated with measures of clinical or sub-clinical atherosclerosis, diabetes or with other components of the metabolic syndrome. There was strong evidence that IGF-II was positively associated with bifurcation intima-media thickness in fully adjusted models additionally controlling for the presence of plaque (0.05 mm; 0.01, 0.09), log HOMA insulin resistance (0.12; 0.05, 0.20) and adverse levels of log HDL-cholesterol (-0.03 mmol/l; -0.05, 0.00), log triglycerides (0.09 mmol/l; 0.04, 0.13), blood pressure (3.33 mmHg; 2.20, 4.46), ankle-brachial pressure index (-0.02; -0.04, -0.00), fat mass % (1.13%; 0.52, 1.75) and waist-hip ratio (0.01; 0.00, 0.02). In contrast, log IGFBP-2 was inversely associated with diabetes (odds ratio: 0.67; 0.44, 1.01), bifurcation intima-media thickness (-0.04 mm; -0.09, 0.00), log HOMA insulin resistance (-0.30; -0.37, -0.24) and adverse levels of log HDL-cholesterol (0.06 mmol/l; 0.03, 0.08), log triglycerides (-0.13 mmol/l; -0.18, -0.09), blood pressure (-2.04 mmHg; -3.24, -0.84), fat mass % (-2.73%; -3.30, -2.16) and waist-hip ratio (-0.04; -0.04, -0.03). Results were generally little altered when further adjusted for body mass index or other components of the IGF system. **Conclusion:** The findings for IGF-I are contrary to some but not all reports; adaptive effects secondary to patho-physiological changes may explain these results. The inverse associations of IGFBP-2 with components of the metabolic syndrome are consistent with an emerging epidemiological literature, while the positive associations of IGF-II are novel epidemiological findings which are in line with animal models. These relationships require study in prospective cohorts to help differentiate between aetiological and adaptive effects.

P1-052

Transgenerational Effects of Prenatal Glucocorticoid Exposure on Growth, Endocrine Function and Behaviour in the Guinea Pig Alice Kostaki, Dawn Owen, Dawn Li, Stephen G Matthews; *Departments of Physiology, Obstetrics & Gynaecology and Medicine, University of Toronto, 1 King's College Circle, Toronto, Ontario M5S 1A8, CANADA.*

Background: Synthetic glucocorticoids (sGC) are currently recommended for mothers at risk of preterm delivery between 24-34 weeks to promote fetal lung maturation. Evidence is emerging indicating long-term effects of repeated courses on endocrine function and behaviour in juvenile and adult offspring. However, very little is known concerning potential transgenerational influences on growth, endocrine function and behaviour. In the present study, we hypothesized that repeated treatment of grandmothers (F_0) with sGC will alter growth, hypothalamo-pituitary-adrenal (HPA) function and stress-related behaviour in F_2 offspring with no manipulation of the F_1 pregnancy. **Methods:** Pregnant guinea pigs (F_0; n=8-10/gp) were subcutaneously injected with betamethasone (Beta; 1mg/kg) or vehicle (Veh) on gestational days 40/41, 50/51 and 60/61 or left undisturbed (U). Animals were allowed to deliver naturally. Adult F_1 female offspring from each group (n=8-10/gp) were mated and F_2 offspring delivered with no further manipulation of the F_1 pregnancy. Growth: Offspring weight and size were carefully monitored (n=8-22/sex/gp). HPA axis: Salivary cortisol was determined before (basal) and at several time points after exposure to a novel open-field, and swim stress (2 episodes). Behaviour: On postnatal day (pnd25), activity was measured during a 30 min period of exposure to a novel open-field environment (Opto-Varimax; Columbus Instruments). **Results:** There was no significant effect of Beta or Veh injection (F_0) on birthweight in F_2 offspring. However, by pnd 10 $Beta_{F2}$ male offspring were significantly ($p<0.05$) heavier than Veh_{F2} and U_{F2}. At pnd25, body length and head length were significantly greater in the $Beta_{F2}$ males compared to the Veh_{F2} and U_{F2} offspring. Salivary cortisol (basal and stress-stimulated) in the $Beta_{F2}$ males and females tended to be lower than Veh_{F2} and U_{F2} at all time points. This was most pronounced following repeated exposure to swim stress (males $p<0.005$, females $p<0.02$). There was a significant effect of grandmaternal (F_0) treatment on open-field activity in male ($p<0.02$) and female ($p<0.05$) F_2 offspring. $Beta_{F2}$ males were significantly less active over the 30 min period, while $Beta_{F2}$ females were significantly more active than Veh_{F2} (males $p<0.04$, females $p<0.05$) and U_{F2} (males $p<0.02$, females $p<0.03$) offspring. There were no significant differences between Veh_{F2} and U_{F2} offspring in any of these measurements. **Conclusion:** Prenatal exposure to sGC (F_0) causes transgenerational programming of growth, adrenocortical function and open-field behaviour in young F_2 offspring. While adrenocortical activity was reduced in both $Beta_{F2}$ female and male offspring, effects on growth and behaviour were sexually dimorphic. The mechanisms involved in the non-genomic transgenerational transmission of these phenotypes following prenatal sGC exposure are currently being investigated. **Supported by:** Canadian Institutes of Health Research (SGM).

P1-053

The Effect of *in utero* Tobacco Exposure on the Fetal HPA Axis Sarah D. McDonald[1], Sherry Perkins[2], William Gibb[2], Joseph Beyene[3], Kellie Murphy[4], Arne Ohlsson[4], Mark Walker[2]; [1]Department of Obstetrics and Gynecology, McMaster University, Hamilton, ON L8N 3Z5 Canada, [2]University of Ottawa, Canada, [3]Hospital for Sick Children, Toronto, Canada, [4]University of Toronto, Toronto, Canada

Background *In utero* "programming" of the hypothalamic-pituitary-adrenal axis may occur if cigarette smoking induces a state of chronic oxidative stress in the fetus. Infants of smokers have lower birth weights. Low birth weight has been associated with raised fasting plasma cortisol concentrations in adults.(Phillips, 2000) We sought to determine the relationship between *in utero* tobacco exposure and cortisol levels in the neonate. **Methods** This was a prospective cohort study of 83 infants of non-smoking mothers and 21 infants of smoking mothers. The primary exposure was self-reported cigarette smoking (dichotomous, "yes" or "no"), which we have validated during pregnancy (correlation coefficient 0.92 between the reported number of cigarettes smoked and cotinine level in pregnant patients).(McDonald, 2005) Healthy women having elective cesarean sections under spinal or epidural anaesthetic

at term (38 weeks and 0 days– 41 weeks and 3 days) with intact membranes and healthy singleton fetuses were invited to participate. Signed informed consent was obtained. Morning, fasting maternal blood samples were collected from the antecubital vein using standard phlebotomy technique. Umbilical arterial and venous cord blood was drawn from the clamped umbilical cord after delivery of the baby. **Results** Baseline characteristics were similar except for maternal age (smokers were significantly younger, 29.4 ± 6.0 years versus 32.6 ± 4.3 years, respectively, p=0.030) and the household income and education levels were less in smokers. Infants of smokers weighted significantly less than non-smokers (3212.6 ± 443.4 g versus 3527.6 g ± 453.2g, respectively, p=0.005). Cortisol levels were similar while ACTH levels were almost double in smoke-exposed infants, even after adjusting for potential confounders.

Table 1 Laboratory values

	Mean (SD) in smokers	Mean (SD) in non-smokers	p value
Umbilical arterial cortisol, nmol/L	197.33 (93.10)	206.16 (90.51)	0.692
Umbilical arterial ACTH, pmol/L	**16.72 (12.92)**	**8.80 (9.66)**	**0.004**
Maternal cortisol, nmol/L	918 (186)	857 (201)	0.207
Maternal cotinine, ng/mL	96.2 (83.4)	0.1 (0.5)	<0.001

Conclusions We have demonstrated that infants exposed to *in utero* tobacco smoke have significantly elevated ACTH levels compared with non-exposed infants, a potential set up for "programming" of the HPA axis. Cortisol levels were not significantly different, which may represent a blunted response of the fetal adrenal gland *in utero*. The elevated levels of umbilical arterial ACTH are most likely due to direct stimulation the fetal HPA axis by nicotine which crosses the placenta, although t is also possible that cortisol is metabolized faster in tobacco-exposed fetuses.

P1-054

Sucrose Overload During Pregnancy and Lactation Influences Body Weight and Blood Pressure in Adult Offspring Daniela A. Mirandola, Luzia N. S. Furukawa, Joel C. Heimann; Nephrology Section of the University of São Paulo School of Medicine, São Paulo – Brazil

Objective: To evaluate the influences of sucrose overload during the perinatal period on blood pressure (BP), body weight (BW) and plasma renin activity (PRA) in adult rats. **Methods**: Female Wistar rats were fed a low (LSD: 0.15% NaCl), normal (NSD: 1.3%) salt diet or NSD and 20% sucrose solution that was given ad libitum in substitution to drinking water (SUC) since 8 weeks of age. At 12 weeks of age, they were matched with males. After weaning, all offspring received only NSD. At 12 weeks of age, plasma glucose, BP and PRA were evaluated. **Results** (mean±SEM): BW (g) was lower ($p<0.05$) at birth in the offspring from SUC dams (females: LSD 6.0±0.10 - n= 63, NSD 6.0±0.06 – n= 80, SUC 5.5±0.09 – n= 61; males: LSD 6.6±0.09 – n= 71, NSD 6.2±0.07 – n= 89, SUC 5.9±0.08 – n= 68) and this difference was maintained until 12 weeks of age. Blood glucose (mg/dL) was higher ($p<0.05$) in the offspring from SUC dams (females: LSD 96±1.7 – n= 25, NSD 100±2.0 – n= 33, SUC 103±1.7mg/dL – n= 29; males: LSD 104±2.3 – n= 32, NSD 108±2.4 – n= 33, SUC 116±2.0 – n= 29). Higher ($p<0.05$) BP (mmHg) was observed in the offspring of SUC dams (females: LSD 105±3.3 – n= 8, NSD 97±4.9 – n= 2, SUC 137±5.1 – n= 6; males: LSD 112±1.6 – **n= 10, NSD 119±4.0 – n= 7, SUC 134±4.5 – n= 3).** Finally, higher ($p<0.05$) PRA (ng/mL/h) was observed in the female offspring from LSD dams (LSD 8.6±0.5 – n= 6, NSD 2.6±0.6 – n= 4, SUC 3.2±0.5 – n= 5). **Conclusions**: Body weight, blood glucose and blood pressure in adult rats are influenced by their mother's sucrose intake during pregnancy and lactation.
Supported by FAPESP.

P1-055

Over Nutrition During Lactation Induces Obesity and Heart Metabolic Programming when the Rats Turn into Adults Anibal S. Moura and Cristiano C. N. Franco de Sá. *Department of Physiological Sciences. Laboratory of Physiology of Nutrition and Development. State University of Rio deJaneiro, Rio de Janeiro, RJ 20550-030 BRAZIL*

Background: Imprinting in early life inducing metabolic program in adulthood have been described in different systems and organs from human and experimental animal. Nowadays strong evidences are showing that obesity and cardiovascular diseases may be due nutritional disturbances at post-natal period. Several hormones are involved in this process. For instance, the adipocyte secreted hormone leptin and the pancreatic beta cell secreted insulin, have been suggested to be directly involved in the control of heart growth and energy use. However, the impairment the normal action and interactions between then still is poorly studied. The objective of this work is to evaluate the interaction of signaling pathways of both hormones in rats overfeed during lactation when they turn into adults, one year after weaning. **Methods**: Virgin females Wistar rats were time mated with normal males at the age of three months. To induce early post-natal over nutrition the primary litter size was adjusted on the third day of life to 3 rats per litter (overfed group; O-group) and control group was set with 10 lactating pups per dam (control group; C-group). The pups from both groups were studied 1 year after weaning. It was measured: animal weigh corporal (g), visceral fat (g); heart, glucose (mg/dL) and plasmatic insulin (uU/ml) (n=6). Using western blotting methodology it was studied in heart left ventricle by the plasma membrane content of: IR (Insulin receptor), ObR (leptin receptor), IRS-1 (Insulin receptor substrate-1), PI3-Kinase (Phosphatidylinoditol 3-kinase p85), JAK-2 (Janus tyrosine kinase-2), STAT-3 (Signal transducer and activator of transcription-3) and GLUT4 (Glucose transporter-4). These proteins were measured after the stimulation of the left ventricle with 1.M of Insulin (Ins), 50 ng/ml of Leptin (Lep) or Insulin+Leptin (Ins and Lep). Immunoreactive proteins were visualized by 3,3′-diaminobenzidine staining. The bands were quantified by densitometry, using Image J Software (NIH, USA).The results were expressed as mean + standard error of mean (SEM) and were statistically analyzed by Student's t-test and ANOVA, using a significance level of $p<0,05$. **Results**: There were significant increase of the corporal weight, visceral fat ($p < 0,05$) and heart weight ($p < 0,05$). Also, it was observed a significant increasing of plasmatic insulin (C =36, 7 ± 5, 7 and O = 67, 8±11) without alteration of the plasmatic glucose. The stimulated hearts revealed that in the group C leptin inhibited the action of the insulin and reduced the GLUT4 expression ($p < 0,05$). In this group was observed also a reduction of IR and IRS-1. On the other hand the JAK-2/STA-3 pathway was found increased. In obese rat heart insulin even with an increasing of IR and IRS-2 was unable to translocated GLUT4. Also, in this group leptin inhibited the insulin action and increased the JAK-2/STAT-3 pathway. **Conclusions**: Insulin and leptin interacts in the cardiomyocyte energy supply modulation. In obese rats the heart develops an insulin resistance due the increased activity of the ObR/JAK-2/STAT-3 pathway. Our data strongly suggest that overfeeding during early life acts as a strong imprinting factor. Support: FAPERJ (E-26/171.604/2004) and CNPq (304018/2004-0)

P1-056

Insulin and Leptin Signaling in Rats' Hearts is Modulated by Nutrition During Lactation Renata O. Pereira, Annie B. Moreira, Laís de Carvalho* and Anibal S. Moura; *Department of Physiological Sciences. Laboratory of Physiology of Nutrition and Development and Department of Histology and Embriology. State University of Rio de Janeiro. Rio de Janeiro. RJ 20550-030 BRAZIL*

Background: Malnutrition during critical developmental periods is suggested to be a risk factor for obesity and associated metabolic disorders in later life. Insulin has been described as a potential mediator of intrinsic responses to nutritional state in the heart due to its effects on cardiac metabolism, mainly on glucose transport. It has been demonstrated that leptin can act through some components of the insulin-signaling cascade. Thus, our aim was to analyze how overfeeding during suckling period alters insulin and leptin signaling, modulating energy metabolism in rat's hearts. **Methods**: Male Wistar rats were divided in two groups, overfed (litter size reduced to only three pups from day 3 to day 10 or 21) and control (normal litter size from birth to day 10 or 21). At 10 and 21 days we analyzed by Western Blotting some key proteins of insulin and leptin cascades: insulin receptor (IR), leptin receptor (ObR), phosphatidylinoditol 3-kinase p85 (PI 3-kinase), Janus tyrosine kinase 2 (JAK2), signal transducer and activator of transcription 3 (STAT3), and the glucose transporter GLUT4. We also assessed pup's weight, the plasma level of glucose, insulin and leptin, as well as insulin/glucose ratio. **Results**: Overfed animals when compared to control animals displayed an increase in body weight about 37% at 10 days and 31.6% at 21 days. Plasma insulin (uU/ml) was also found increased in overfed animals (10 days old = 32.48±2.31; 21 days old = 31.03±0.61) compared to control group (10 days old = 25.22±1.28; 21 days old = 19.56±0.83). However, leptin (ng/ml) was not affected by development but only by nutrition showing increased concentration in overfed animals (Control = 2.8±0.5 and overfed =4.5±1.2). Also, it was observed at 21 days a higher insulin/glucose ratio in overfed animals (3.18±0.27) when compared to control (1.92±0.08). Regarding to Western Blotting analysis our data demonstrated that there was a time-dependent enhancement in the content of all components of both insulin and leptin cascades analyzed, except for STAT3. In addition, we observed that overfeeding lead to an increase of PI3-Kinase content at 10 days and of IR, ObR and STAT3 content at 21 days. Moreover, GLUT4 translocation was affected by both animal development and overfeeding. **Conclusions**: Our data showed that overfeeding affected animal body weight and plasma leptin and insulin and increased insulin/glucose ratio, indicating basal insulin resistance. However, in the heart we observed a high sensitivity to insulin, demonstrated through the activation of insulin cascade, mainly at 21 days. On the other hand, we also demonstrated an activation of leptin signaling. We suggest that overfeeding triggered a synergetic effect of insulin and leptin on heart, leading to an improved heart energy supply.
Support: FAPERJ (E-26/171.604/2004) and CNPq (304018/2004-0)

P1-057

Placental Restriction Impairs Insulin-like Growth Factor (IGF) Action on Glucose Metabolism but Enhances that on Lipolysis and Adipose Tissue Postnatally in the Sheep Miles J De Blasio, Melissa R Walker, Robyn L Taylor, Patricia A Grant, Kathryn L Gatford, Phillip C Owens, Jeffrey S Robinson, Julie A Owens. Department of Obstetrics and Gynaecology, University of Adelaide, Adelaide, South Australia, Australia, 5005.

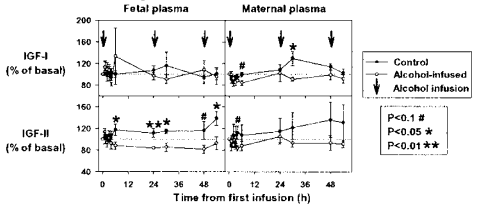
Background: The insulin-like growth factor (IGF) axis influences insulin action and glucose homeostasis through multiple pathways. These include modulation of glucose uptake, insulin secretory capacity and body composition. Small size at birth reflecting an adverse prenatal environment is associated with impaired glucose tolerance and diabetes, and reduced circulating IGF-I has been implicated as contributing to this. Here the effect of placental restriction of fetal growth on circulating IGFs and

metabolic sensitivity to IGF-I in the young lamb was determined. **Methods**: Placental growth was restricted (PR) by removal of the majority of endometrial caruncles in ewes prior to mating. Size at birth was measured in the offspring of these ewes (placentally restricted lambs, n=15) and in control lambs (n=19) and after overnight fasting, a hyper-IGF-I euglycaemic clamp (3μg/kg/min) was performed at 40 days of age to measure glucose and free fatty acid (FFA) sensitivity to IGF-I. Fasting plasma IGF-I and IGF-II were measured by specific radioimmunoassays following size-exclusion HPLC at pH 2.5 to remove IGFBPs. **Results**: PR reduced size at birth in terms of weight (-27%), crown-rump length (-11%), various long bone lengths (-8 to -12 %), and abdominal circumference (-10%) ($p<0.05$). PR also increased fractional growth rate in terms of weight, metatarsal length and shoulder height were increased by x to y% ($p<0.05$ for all). PR increased fasting plasma glucose (+20%) ($p<0.05$). PR did not alter plasma IGF-I and IGF-II, but these decreased with decreasing size at birth in terms of weight ($r=0.72$, $p<0.01$), tibia length ($r=0.67$, $p<0.05$). PR reduced IGF-I sensitivity of glucose metabolism (-45%, $p<0.05$), which also decreased with increasing thinness at birth in terms of ponderal index ($r=0.73$, $p<0.025$). IGF-I sensitivity of FFA correlated positively with visceral fat mass in placentally restricted lambs ($r=0.58$, $p<0.05$). **Conclusions**: In summary, placental restriction of fetal growth reduces circulating insulin-like growth factors and sensitivity of glucose metabolism to IGF-I, which may contribute to impairment of insulin secretory capacity and glucose homeostasis. The latter may be further exacerbated by expansion of visceral adipose tissue, driven in part by enhanced IGF-I sensitivity of FFA following placental restriction.

P1-058

Moderate Fetal Alcohol Exposure in Late Gestation Reduces Fetal IGF-II Abundance and Alters Circulating IGFBPs KL Gatford[1], JA Owens[1], PA Dalitz[2], ML Cock[2], R Harding[1]; [1]Department of Obstetrics & Gynaecology, University of Adelaide, Adelaide SA 5005, Australia; [2]Department of Physiology, Monash University, Melbourne VIC 3800, Australia

Background: Repeated acute maternal ethanol (EtOH) treatment, mimicking 'binge drinking', causes a near complete cessation of growth in the fetal sheep. Insulin-like growth factor-I and -II (IGF-I and IGF-II) in the fetus regulate growth and in the mother, affect substrate partitioning and their placental delivery to the fetus. We therefore hypothesised that decreased circulating IGFs might mediate some of the effects of acute alcohol exposure on reduced fetal growth. **Methods:** EtOH (1 g/kg maternal weight, n=8) or saline (n=6) was administered to twin-bearing, chronically catheterised ewes for 1 h on 3 consecutive days at 0.8 of gestation (Figure, arrows). Fetal and maternal plasma were collected prior to and 6 h after infusions each day, and also 1, 2, 3 and 4 h after infusion on the first day. Fetuses were weighed at postmortem two days after the final infusion. Plasma was subjected to size-exclusion HPLC at pH 2.5. IGF-I and –II were measured by specific RIA in fractions containing free IGFs, and analysed by repeated measures ANOVA. Plasma IGFBP was analysed in plasma pools from control and EtOH-treated mothers and fetuses by Western ligand blot and autoradiography after probing with ^{125}I-IGF-II. **Results:** Maternal alcohol treatment reduced fetal body weight (Control: 2.66 ± 0.16 kg; EtOH: 2.13 ± 0.12 kg; $p=0.02$), but did not change fetal plasma concentrations of circulating IGF-I ($P=0.97$, Figure). Fetal plasma IGF-II, relative to pre-infusion levels, was lower in EtOH-treated fetuses than in control fetuses ($P=0.034$, Figure). Maternal plasma IGF-I, relative to pre-infusion levels (Figure), changed differently with time in control and EtOH-treated ewes ($P=0.043$), such that maternal plasma IGF-I was lower in EtOH-treated than in control ewes at 6 ($P=0.055$) and 30 ($P=0.013$) hours after the start of the first infusion. Maternal plasma IGF-II was not affected by treatment ($P=0.30$, Figure). EtOH reduced IGFBP-3 abundance and increased that of IGFBP-2 in pooled maternal plasma of EtOH-treated compared to control ewes.



Conclusions: Maternal alcohol exposure reduced fetal IGF-II by 28.5% and maternal IGF-I by 15% between 24 and 54 h after the first infusion and altered maternal IGFBPs, which may act via several mechanisms to cause some although not all of the fetal growth restriction observed. Alcohol induced disturbances of the maternal and fetal IGF axes may be a relatively common initiator and pathway of programming of later dysfunction in offspring.

P1-059

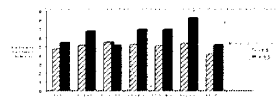
Patterns of Growth from Birth to Adulthood, Adiposity and Cortisol Secretion in Mid-adulthood Chris Power[1], Leah Li[1], Clyde Hertzman[2]. [1]Centre for Paediatric Epidemiology and Biostatistics, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK. [2] Human Early Learning Partnership, UBC, Vancouver, Canada.

Background. Many studies have investigated the role of pre and postnatal growth and of obesity on chronic disease in later life. Attention also now focusses on biological processes, including function of the hypothalamic-pituitary-adrenal (HPA) axis that may link early growth, adiposity and chronic disease. One hypothesis is that the HPA axis can be altered early in life with long-lasting effects on cortisol regulation. We examine whether pre and postnatal growth is associated with cortisol secretion in mid-adulthood and whether obesity and central adiposity also affect secretion patterns. We are concerned with the timing of associations, whether growth and adiposity in early life are related to later cortisol secretion through a link with adult body size or whether they have a separate contribution. **Methods.** We examined prospectively collected data on birthweight, gestational age, head circumference at birth, childhood height and BMI (7y) and adult height, BMI (33y) and WHR (44y) from the 1958 British birth cohort in relation to salivary cortisol at age 44y (n=6335). Two saliva samples were collected on the same day: 45mins post-waking (t1) and 3 hours later (t2). Four outcomes were constructed to differentiate secretion patterns (1) post-waking cortisol, (2) slope (t1 to t2), (3) area-under-curve (AUC), and (4) pattern of change t1-t2 (decline, rise, low flat, other flats). **Results**. Men had a lower t1 cortisol (21.01 nmol/l), but a higher t2 level on average (9.2 nmol/l), than women (21.88 and 8.24 nmol/l, respectively). As BMI at 7y and adult WHR increased for men, post-waking (t1) cortisol levels decreased; among women, as head circumference, height and WHR increased, t1 decreased. 84.2% of men and 88.0% of women had a 'normal' decline pattern: a t1 level >10nmols/l and a decline to t2 of >20%. Increasing BMI at 7y and adult WHR both predicted a decrease in the proportion with a 'normal' decline in men; while increasing WHR was also associated with a decreasing proportion of 'normal' decline among women. For men, the steepness of the negative slope (t1-t2) diminished with increasing BMI at ages 7y and 33y, and with WHR at 45y. For women, as height at 7y and 33y increased the steepness of the negative slope also diminished. AUC was influenced by prenatal factors (for men, birth-weight for gestational age and for women, head circumference at birth), childhood growth (height at 7y), and adult factors (BMI at 33y, WHR at 45y for men only, and height at 33y for women only). All except birth-weight for gestational age were negative associations. **Conclusions:** WHR emerged as a major factor among men and there was evidence that adult adiposity (WHR and 33y-BMI) was also important for women. Also early childhood measures were important for males (BMI) and for females (head circumference and height), in particular for AUC. Adiposity is associated with cortisol secretion patterns in mid-adulthood and this is all the more important given the increasing levels of adiposity in this population and elsewhere.

P1-060

Maternal Consumption of a High-meat, Low Carbohydrate Diet in Late Pregnancy and Stress Responsiveness in the Offspring [1]Rebecca M Reynolds, [2]Helen Simonsen, [2]Sharon Pearson, [2]Mary Barker, [2]David JP Barker, [2]Mary Campbell-Brown, [2]Keith M Godfrey, [2]David IW Phillips; *[1]Endocrinology Unit, University of Edinburgh, Queen's Medical Research Institute, Edinburgh, UK; [2]Medical Research Council's Epidemiology Reource Centre, Southampton. UK*

Background: Consumption of a high-meat, low carbohydrate diet in late pregnancy is associated with fetal growth restriction, and raised blood pressure/altered glucose tolerance in the offspring. As high protein diets stimulate the hypothalamic-pituitary-adrenal (HPA) axis, we hypothesised that an unbalanced diet increases maternal cortisol levels, exposing the fetus to excess cortisol and programming the offspring's HPA axis. In a preliminary study in Motherwell, Scotland, where pregnant women had been advised to eat one pound (0.45kg) of red meat per day during pregnancy and to avoid carbohydrate-rich foods, we found elevated fasting plasma cortisol levels in men and women whose mothers reported higher protein consumption in pregnancy. These elevated cortisol concentrations may reflect an enhanced stress response. As the Motherwell recommendations have similarities to the Atkins diet followed by many women today, the aim of the current study was to test our hypothesis in this cohort by measuring the change in cortisol secretion in response to a psychological stress test (The Trier Social Stress Test (TSST)). **Methods**: 86 subjects born in Motherwell, Scotland, during 1967-68 were invited by letter to re-attend an afternoon clinic for a TSST. Subjects taking systemic glucocorticoids in the previous 3 months were excluded. They underwent a series of stress tasks in front of 2 people and a video camera including a 3 min mental arithmetic test and a public speaking task for 5 min. Before and after each stress test blood pressure was recorded, subjects collected saliva samples and venous blood was sampled. Saliva and plasma samples were stored at –80C for subsequent measurement of cortisol. After the TSST subjects completed a short questionnaire about their current medication and any changes in their health since last visited. Body composition was assessed by calculating BMI and measuring waist/hip ratio and bioimpedence. **Results**: In response to the stress test there were significant increases in BP and heart rate in both men and women. The greatest change in BP or heart rate was in response to the public speaking challenge (BP increase 14mmHg, $t=-4.5$, $p<0.0001$; HR increase 6bpm, $t=-2.35$, $p=0.02$). Both BP and heart rate fell to baseline values by the end of the recovery period. Plasma cortisol also rose in response to stress (men 371 to 478 nmol/l, $t=-5.1$, $p=0.00002$; women 348 to 380 nmol/l, $t=-2.2$, $p=0.03$). Between early and late pregnancy, meat consumption almost doubled while carbohydrate intake fell to a third. Offspring of mothers who reported greater meat intake during late pregnancy had greatest cortisol response to stress ($p=0.03$) (See Figure). **Conclusions**: The TSST is a robust method in our hands demonstrating significant changes in blood pressure, heart rate and cortisol in response to stress. Although the specific advice given to mothers in this study precludes direct application to other populations this is the first evidence that an unbalanced maternal diet during late pregnancy influences stress responsiveness in the offspring. These findings add to



increasing evidence that adverse maternal factors program lifelong effects in the offspring.

P1-061

Repeated Maternal and Fetal Glucocorticoid Exposure During Baboon Pregnancy – Effects on Maternal and Fetal Leptin and Metabolism

Natalia Schlabritz-Loutsevitch[1,2], Juan Carlos Lopez-Alvarenga[2], Kyle Peebles[2], C. Jett[2], A.G. Comuzzie[2], Susan Jenkins[1] and Peter Nathanielsz[1,2]; [1]*Center for Pregnancy and Newborn Research, Department of Obstetrics, University of Texas Health Sciences Center, San Antonio, Texas;* [2]*Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, Texas 78227*

Background: Glucocorticoid (GC) administration during pregnancy has metabolic effects on mothers and their fetuses. Leptin is an important regulator of energy homeostasis. We investigated short- and long-term consequences of repeated courses of maternal GC administration on maternal and fetal metabolic parameters and leptin in the baboon.
Methods: 25 pregnant baboons received either prenatal BM (bethamethasone; 175 $\mu g \cdot kg^{-1} \cdot d^{-1}$ at 111-112, 118-119, 126-127 days gestation (dGA); n=13) or saline (controls -CTR; n=12) i.m. Maternal biochemical and hematology parameters, body weight, and serum leptin were measured after each injection and 144 and 165 dGA. At 175 dGA l c-section and fetal necropsy were performed. Quantitative RT-PCR for leptin was performed on placental mRNA.
Results: Maternal glucose decreased by 32% by 175 dGA compared with initial level in BM mothers (p=0.001; Fig 1B), but was not significantly changed in CTR mothers. Cholesterol and leptin rose similarly in CTR and BM mothers (Fig. 1A and C). Fetal organ and placental weight were not different at 175 dGA in both groups. Leptin level in amniotic fluid (2.9 ± 0.9 ng/ml), fetal and maternal circulations (1.5 ± 0.4 ng/ml and 89.1 ± 33 ng/ml, respectively) and placental leptin mRNA levels were similar in CTR and BM groups. The maternal:fetal serum leptin ratio correlated positively with placental diameter in BM-treated (r=0.96, p<0.02), but not in CTR groups (Fig. 1D) and was lower in BM-treated compared to CTR (53.3 ± 4.3 vs 66.7 ± 4, $p < 0.025$).
Conclusions: In contrast to results obtained in the rat, maternal GC treatment of pregnant baboons suppressed the maternal:fetal leptin ratio. The decrease in the maternal:fetal leptin ratio may indicate long-term metabolic effects of prenatal GC exposure on the placenta.

P1-062

IGF-I Function Is Depressed In Fetuses Of Pregnant Baboons Fed 70% Of The Global Diet Of *Ad Libitum* Fed Baboons During The First Half Of Gestation

N. E. Schlabritz-Loutsevitch,[3], M. Levitz[2], G. B. Hubbard[1], S. L. Jenkins[3], L. A. Cox[1], C. Li[3], Victor Han[4], T.J. McDonald,[3] and P.W. Nathanielsz.[3] [1]*Southwest Foundation for Biomedical Research, San Antonio, Texas 78227;* [2]*Department of Obstetrics, New York University School of Medicine, New York, New York 10016;* [3]*Center for Pregnancy and Newborn Research, Department of Obstetrics, University of Texas health Sciences Center, San Antonio, Texas and* [4]*Children's Health Research Institute, University of Western Ontario, London, Canada N6C 2V5*

Information on the influence of maternal nutritient restriction on the regulation of placental and fetal growth and development is critical to a better understanding of the physiology and pathophysiology of pregnancy. While the effects of a wide range of controlled maternal nutrient restriction (MNR) during pregnancy have been extensively studied in rodents and sheep, there are no data available on the effects of controlled and monitored MNR on non-human primate placental and fetal development. The goal of this study was to determine the effects of carefully controlled and monitored MNR in a well-studied non-human primate species, the baboon, on important hormonal indices of fetal growth. **Methods:** MNR baboons were given 70% of the food consumed by a*d libitum* fed control (CTR) mothers from 30 to 90 days gestation (dGA). **Results:** CTR mothers (n=8) weighed 13.4 ± 0.6 kg and 13.61 ± 0.4 kg at 30 and 90 dGA respectively. MNR mothers (n=6) weighed 13.0 ± 0.2 and 12.2 ± 0.3 kg at the same stages. The fall in maternal weight at 90 dGA ($p<0.05$) and represents 5.5% weight loss. Fetal weight in the MNR group at 90 dGA was 95.4 ± 3.3 g (n=6) compared to 100.9 ± 3.4 g (n=8) for fetuses of control-fed mothers. This difference, a 9.5% reduction in fetal weight was not statistically significant. A power analysis of the individual data indicated that with 44 animals in each group with a power of 0.8, there would have been a significant difference in fetal weight at the $p<0.05$ level. Ratio fetal/placenta weight was essentially the same in both groups. Fetal to maternal serum glucose ratio was also depressed by MNR (p=0.05). Although body weight was not significantly reduced in either male or female fetuses, femur length was decreased in female fetuses of mothers exposed to MNR ($p<0.05$). Umbilical cord length was also decreased in both sexes ($p<0.05$). This degree of global MNR decreased IGF-I in the fetal circulation associated with decreased IGF-I/IGFBP3 ratio in the fetal circulation. ($p<0.05$). Gene array analysis of the fetal liver demonstrated decreases in expression of IGF-I and IGFBP-6 genes, increases in expression of IGFBP-1, -2 and –4 genes, and no change in IGFBP-3 gene. *In situ* hybridization of liver and placentae from CTR and MNR fetuses demonstrated expression of IGF-I, IGF-II and IGFBP-1 mRNAs in the hepatocytes and IGFBP-3 mRNA in the Kupffer's cells of the liver, and IGF-I and IGF-II mRNAs in the villous and extravillous cytotrophoblasts, IGFBP-3 mRNA in the chorionic mesoderm, and IGFBP-1 and IGFBP-3 mRNAs in the decidua. Hepatic expression of IGF-I decreased, whereas expression of IGFBP-1 increased.
Conclusions: These findings show that even moderate MNR results in decreased activity of the IGF axis in the fetal primate that may be one of the underlying mechanisms of fetal adaptive responses to decreased nutrient supply which conserves energy and potentially plays a role in developmental programming.

P1-063

Gynaecological Disturbances Among Females Engaged in the Manufacture of Sex Hormones Mona Siha, Egypt

Introduction and objective: Numerous studies have established as association between exposure to sex hormones and many gynaecological troubles. The aim of this work is to investigate the different gynaecological disturbances which may affect female workers occupationally engaged in the manufacture of contraceptive pills and other hormonal preparations. **Materials and methods**: The total number of female workers was 214, a control group of 220 subjects was taken of comparable age and socioeconomic status and not exposed to any external source of hormones. All workers were subjected to a prepared questionnaire including present, past, family and occupational history. Gynaecological examinations were carried out for married female workers who agreed to cooperate with the team (137 exposed, 180 control). Virgins were excluded. **Results and discussion:** This study showed the presence of masculinizing signs among the exposed group. There was also positive statistically significant difference between exposed and control on comparing gynaecological disorders .Hysterectomy was done to 11.2% of exposed workers versus 3.6% of non-exposed workers. Our study showed a significant positive relationship between duration of exposure and the prevalence of hysterectomy cases. About 51% of married exposed workers had reproductive disorders .Gynaecological examination showed that exposed workers suffered from vulvo-vaginitis (46.7%), cervical erosion (3.9%) and leucorrhea (62.8%) ($P<0.05$). About 12% of the exposed workers complained of some family health disturbances in the form of precocious puberty in female children, gynaecomastia in male children and husbands. **Recommendations:** We recommend health education for exposed workers for the importance of the use of protective equipments and enclosure of machines. Periodic medical examination should be carried out regularly for early detection of affected personnel.

P1-064

Alterations in Factors Regulating Follicular Development in Sheep Offspring Exposed to Prenatal Betamethasone Deborah M Sloboda[1,2], Yordanos Tesfai[1], Ray J Rodgers[3], Helen F Irving-Rogers[3], Roger Hart[1]; [1]*School of Women's and Infants' Health, The University of Western Australia, Perth, WA;* [2]*Women and Infants Research Foundation, Subiaco, WA;* [3]*The Research Centre for Reproductive Health, The University of Adelaide, SA.*

Background: Although postnatal reproductive development and function have been linked to reduced fetal growth and prenatal events, the mechanisms underpinning this relationship remain unclear. We have previously shown that prenatal exposure to maternal glucocorticoids in sheep results in long-term changes in a number of endocrine axes. To determine whether prenatal glucocorticoid exposure may also influence ovarian follicular development, we examined the effects of maternal administration of betamethasone late in gestation on protein expression in ovaries collected from post-pubertal lambs at 8.5 months of age. ***Methods:*** Pregnant ewes carrying singleton fetuses were injected with saline or betamethasone (0.5mg/kg) at 104, 111 and 118 days of gestation (d). Ewes delivered spontaneously and lambs were weaned at 3 months of age. In females at 8.5 months of age, blood samples were taken for endocrine measures and ovaries were weighed and either frozen (right ovary) or fixed (left ovary) (saline n=5; betamethasone n=3). Growth differentiation factor-9 (GDF-9, transcription factor regulating follicular development) was measured by Western blot; glucocorticoid receptor (GR) was localised by immunohistochemistry; and insulin-like growth factors (IGF), insulin and luteinising hormone (LH) were measured by RIA. Primordial and primary follicles were counted in serial sections. ***Results:*** Lamb weight was similar between groups at 8.5 months of age but left ovary weight was significantly reduced in betamethasone exposed offspring. Maternal betamethasone administration late in gestation significantly elevated plasma IGF1 and IGF2 ($P<0.05$) but not insulin in post-pubertal offspring. LH levels were elevated in betamethasone exposed offspring but differences were not significant. Primary follicle number in the left ovary (3037 ± 700) of betamethasone treated animals was increased compared to controls (1849 ± 395) but did this difference did not reach statistical significance. GR staining was apparent in both granulosa and stromal cells in the ovary cortex. GF staining intensity was increased in betamethasone exposed offspring. GDF-9 protein levels were increased in betamethasone exposed animals by 36%, but this difference was not statistically significant ($P=0.053$). ***Conclusions:*** Exposure to maternal betamethasone late in gestation resulted in a significant increase in circulating IGFs, moderate changes in LH and a modest increase in ovarian primary follicle number and GDF-9 and GR protein levels in post-pubertal offspring. This is the first study to suggest that exposure to prenatal glucocorticoids late in gestation may result in alterations in the expression of factors that control follicular development. These observations suggest a potential for altered reproductive function later in life. Our observations resemble characteristics of The Polycystic Ovarian Syndrome and may have serious implications for women who received antenatal glucocorticoids *in utero*.

P1-065
Prenatal Programming of Hypertension: Role of Maternal Glucocorticoids
Lori L. Woods and Wei Wei; *Division of Nephrology and Hypertension, Oregon Health & Science University, Portland, OR 97239 USA*

Background: Maternal glucocorticoids have been postulated to play an important role in prenatal programming for adult hypertension in the offspring. However, we have shown previously that offspring hypertension caused by maternal dexamethasone administration at 100 µg/kg/d sc can be accounted for by the corresponding reduction in food intake that these mothers experience. The present studies were designed to determine whether there is a lower dose of dexamethasone that does not reduce maternal food intake yet still causes hypertension in the adult offspring. Methods: We treated pregnant rats with dexamethasone at 50 (D50) or 25 (D25) µg/kg/d sc on days 15-20 of gestation. We left an additional group untreated or gave them vehicle injections (control, C). In adulthood (~21 wks), we chronically instrumented male and female offspring for physiological measurements. **Results**: D50 dams reduced their food intake by 22% during and after treatment and gained 35% less weight than C over the course of gestation; D25 dams ate a normal amount of food and their weight gain was not significantly different from that of C. Average birth weights in both D50 and D25 were reduced. However, adult male and female offspring of D50 and D25 had normal mean arterial blood pressures (MAP).

	C	D50	D25
Maternal food intake, d 15-22 (g)	171˜3	133˜5*	157˜9
Pregnancy weight gain (g)	144˜12	93˜9*	123˜13
Birth weight (g)	6.27˜0.18	5.00˜0.15*	5.21˜0.29*
Adult offspring MAP (mmHg)			
Males	127˜2	131˜1	127˜3
Females	120˜2	121˜2	117˜2

(*p<0.05 compared to C)

Conclusions: There does not appear to be a dose of dexamethasone that programs offspring hypertension without reducing maternal food intake and weight gain. These data provide further evidence that maternal glucocorticoids do not program offspring hypertension directly, but that the programming effect of maternal dexamethasone administration is indirect, mediated by changes in maternal nutrition.

Neurologic or Mental Health Outcomes

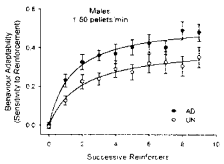
P1-066
Effects of Fetal Growth Restriction on Risks of Low Intellectual Performance are Modified by Gestational Age Bergvall N [1], Iliadou A[1, 2], Johansson S [1], Tuvemo T [3], Cnattingius S [1]. [1]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden. [2]Unit of Clinical Epidemiology, Department of Medicine, Karolinska Institutet, Stockholm, Sweden. [3]Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden

Background: Intellectual performance and cognitive function are independently associated with both gestational age and measures of fetal growth. It has however been hypothesized that the impact of fetal growth restriction on the child's long term cognitive and motor development may vary with gestational age. **Methods**: Population-based cohort study of 352 125 men born between 28 and 43 completed weeks of gestation from 1973 to 1981 in Sweden and conscripted for military service between 1991 and 2000. Studied outcome was intellectual performance at military conscription. Fetal growth was measured by birth weight, birth length and head circumferences, standardized according to Swedish standards and expressed in standard deviation scores for gestational age (SDS). Odds ratios (OR) were calculated to estimate relative risks, using 95 % confidence intervals (CI). Multivariate analysis included besides the measures of fetal growth, adjustment for growth in height between birth and conscription, BMI at conscription, year of conscription, mother's age at delivery, parity, and household's highest socio-economic status, household's highest education and household's family structure during childhood. **Results**: Compared to men born preterm (28-36 weeks) with appropriate birth weight for gestational age (-1 to 1 SDS), men born preterm with a low birth weight for gestational age (< -2 SDS) were not at increased risk of low intellectual performance in young adulthood (OR=0.77; 95 % CI 0.48–1.22). In contrast, men born preterm with a short birth length or a small head circumference for gestational age faced a near doubled risk of low intellectual performance compared to their appropriate peers (OR=1.88; 95 % CI 1.23–2.86, and OR=1.93; 95 % CI 1.37–2.73, respectively). Among men born at term (37-41 weeks), all three measures of fetal growth restriction where associated with increased risks of low intellectual performance: very low birth weight for gestational age (OR=1.15; 95 % CI 1.05–1.27), short birth length for gestational age (OR=1.32; 95 % CI 1.12–1.47), and small head circumference for gestational age (OR=1.24; 95 % CI 1.15–1.33). Being born post-term and with a very low birth weight or very short birth length for gestational age was associated with increased risks of low intellectual age (OR=1.36; 95 % CI 1.08–1.71 and OR=1.43; 95 % CI 1.17–1.76, respectively), whereas being born with a very small head circumference was not associated with increased risks of low intellectual performance (OR=0.96; 95 % CI 0.75–1.23). **Conclusions**: The present study concludes that impaired fetal growth increases the risk of low intellectual performance across all stages of gestation. However, it appears that during early stages of gestation, skeletal growth and brain volume (as measured by birth length and head circumference for gestational age) is more important for intellectual development than birth weight for gestational age. As the time of onset of fetal growth restriction may influence the long-term prognosis of intellectual performance, we suggest that future studies within this field consider also including other dimensions of size at birth, such as birth length and head circumference for gestational age.

P1-067
Maternal Nutrition during Pregnancy Influences Learning and Behavioral Adaptation in Offspring Bernhard H. Breier[1], Christian U. Krägeloh[1], Nichola M. Thompson[1], Jason Landon[1], Jennifer L. Miles[1], Mhoyra Fraser[1] and Michael Davison[1,2] *National Research Centre for Growth and Development and [1]Liggins Institute, Faculty of Medical and Health Sciences and [2] Psychology Department, The University of Auckland, Auckland, New Zealand.*

A substantial body of research, both human and animal, suggests that prenatal influences can have significant later effects on the behavioral and cognitive development of offspring. However, there has been little agreement across experimental reports on the nature and size of the behavioral effects, and little consistency between the results. The general scientific aim of our research program is to investigate whether prenatal influences can shape cognitive function and lifestyle choices during later life. In the present study, we used quantitative procedures to assess learning and behavioral adaptation to changing conditions in a simple model of prenatal undernutrition in the rat (1, 2). Six male offspring of dams undernourished throughout pregnancy (UN offspring) were compared with eight male offspring of *ad-libitum* fed dams (AD offspring). Starting at an adult age of 60 days, offspring had daily behavioral sessions in which they chose between two response alternatives. The procedure of Davison and Baum (3) was used in which the frequencies of rewards for responses on two levers were changed unpredictably throughout the sessions. The time of every experimental event was recorded, and data from the last 35 sessions were analyzed to determine the extent to which choice changed in response to modifications in the relative rate of rewards. Learning and behavioral adaptation, measured as sensitivity to reinforcement, increased significantly ($p<0.001$) with successive pellet deliveries and was on average 30% lower ($p<0.001$) in UN offspring in comparison to AD offspring. Our study shows that offspring of undernourished mothers utilize current environmental information less effectively in determining their choices. They were less adaptable to environmental change and learned less.

Fig 1. Learning and behavioral adaptability (sensitivity to reinforcement) as a function of successive rewards for AD (filled circles) and UN (open circles) offspring. The overall rate of reward supply was 1.5 pellets per min lasting 50 sessions. Sensitivity values were significantly lower in UN offspring than in AD offspring (repeated measures ANOVA, $p<0.001$), and increased with successive rewards delivered ($p<0.0001$). Error bars are + 1 SEM.



Using quantitative behavioral procedures we have shown that simple biological manipulations during fetal development can change choice and learning during later life. In humans, such long-lasting shift in how choices are made will have wide-ranging effects throughout lifespan and may explain the reduced cognitive function and psychiatric sequelae associated with low birth weight or the problematic lifestyle factors that lead to obesity and related disorders.

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P1-068
Psychiatric Outcomes Over Forty Years Following Adolescent Depression and Anxiety Ian Colman, Michael E.J. Wadsworth, Tim J. Croudace, Peter B. Jones. *Department of Psychiatry, University of Cambridge, Cambridge, United Kingdom: Medical Research Council National Survey of Health and Development, Department of Epidemiology and Public Health, University College London Medical School, London, United Kingdom.*

Background: The proposition that adolescents with depression or anxiety are likely to suffer from poor mental health as adults is based largely on clinical samples and relatively short follow-up. Less is known concerning long-term outcomes at the population level. The objective of this study was to define the psychiatric outcomes of common mental disorders in adolescents up to age 53 years. **Methods**: Data from the Medical Research Council National Survey of Health and Development (the 1946 British birth cohort) was used. Assessments of common mental disorders (CMD) were made on 3,279 survey members at ages 13 and 15 years. Survey members were followed-up for numerous psychiatric outcomes in adulthood, including: the prevalence of common mental disorders assessed by the Present State Examination (age 36), the Psychiatric Symptom Frequency Scale (age 43) and the General Health Questionnaire 28 (age 53), self-reported trouble with nerves, suicidal behaviour, and treatment for psychiatric disorder (at ages 26 - 53). Adolescents with CMD at both age 13 and 15 and adolescents with CMD at one of the two ages were compared to mentally healthy adolescents across the outcomes in adulthood. In addition, the

proportion of adult CMD attributable to adolescent illness was calculated. **Results**: Over half the adolescents with CMD at both age 13 and 15 also had CMD at age 36, 43, or 53, more than mentally healthy adolescents (odds ratio=3.5, 95% confidence interval: 1.2, 10.3). They were also more likely than healthy adolescents to have self-reported nervous trouble and to be treated for psychiatric disorder during adulthood. None of these effects was apparent for adolescents with only one episode of CMD at either 13 or 15. Only 5.1% of cases of adult CMD could be attributed to adolescent CMD. **Conclusions**: The long-term psychiatric outcome of persistent or recurrent common mental disorders in adolescents was poor, while the outcome of a single episode was better than expected. The reported association between adolescent CMD and poor psychiatric outcomes in adulthood may be modified by persistence or severity of symptoms in adolescence. Only a minority of adult disorder could be attributed to adolescent disorder.

P1-069

Chronic, Low Levels of Endotoxin Exposure Alter Fetal Brain Development and the Placenta in the Absence of Hypoxemia SM Rees[1], JR Duncan[1], ML Cock[2], K Suzuki[2], J-P Y Scheerlinck[3], R Harding[2]. *[1]Dept of Anatomy and Cell Biol, University of Melbourne, Melbourne, VIC, Australia; [2]Dept of Physiology, Monash University, Melbourne, VIC, Australia; [3]Centre for Animal Biotechnology, University of Melbourne, Melbourne, VIC, Australia.*

Background: Intrauterine infection has been linked to brain injury in human infants although the mechanisms are not fully understood. We recently showed that repeated acute exposure of preterm fetal sheep to bacterial endotoxin (Lipopolysaccharide, LPS) results in fetal hypoxemia, hypotension, increased systemic proinflammatory cytokines and brain damage including white matter injury. However, it is not clear whether this injury is caused by reduced cerebral oxygen delivery or inflammatory pathways independent of hypoxia. In order to resolve this problem, we have used a model of chronic, low-level LPS exposure that does not induce significant fetal hypoxemia. **Objective:** Our aim was to determine the effects on the fetal brain of a chronic intrauterine inflammatory state, induced by LPS infusion into the fetal circulation, a model which does not cause fetal hypoxia. As it is possible that the placenta is affected by LPS we have also analysed placental structure. **Methods:** At 0.65 of gestation (term ~ 147 days) 8 catheterised fetal sheep in utero received i.v. infusions of LPS (5-15 μg) over 5 days; control fetuses received saline. Fetal physiological responses were monitored throughout the infusion. Fetal brain and placental tissues were examined histologically six days after the conclusion of the infusion. **Results:** The low level LPS exposures did not result in physiologically significant alterations to fetal blood gases or mean arterial pressure; however, plasma proinflammatory cytokine levels were elevated. Following LPS exposure there was no difference in fetal body or brain weights ($p>0.05$); placental weight was reduced ($p<0.05$), consistent with reduced placentome cross-sectional area ($p<0.05$). In the fetal cerebral hemispheres subcortical white matter injury was present in 6 LPS-exposed fetuses and included axonal damage, microgliosis, oligodendrocyte injury and increased β amyloid precursor protein (β-APP) expression. Following chronic LPS exposure placental weight and placentome-cross sectional area were reduced and there was modest placental damage. These alterations may result in placental dysfunction during the remainder of gestation; however fetal metabolic status and growth were not adversely affected over the experimental timeframe. **Conclusions:** Chronic, systemic exposure of the fetus to LPS resulted in fetal brain damage in the absence of hypoxemia or hypotension, although the resulting injury was less severe than following repeated acute exposure. As with acute episodic LPS exposure, the placenta was also adversely affected by chronic LPS exposure.

P1-070

Gestational Age and Body Size at Birth Predict Cognitive Abilities at 56 Months of Age among Children Born at Term Kati Heinonen, Katri Räikkönen, Aulikki Lano; Department of Psychology, 00014 University of Helsinki, Helsinki, Finland, Department of Pediatric Neurology, Hospital for Children and Adolescents, 00029 Helsinki University Central Hospital, Helsinki, Finland

Background. Individual differences in cognitive ability may in part have prenatal origins. In premature babies gestational age and body size at birth correlate positively with later cognitive test scores. However, less is known whether this holds for those born at term (37-42 weeks' gestation). The purpose of this study was to examine in a prospective study whether gestational age and body size at birth (weight, length, head circumference, ponderal index and head/length-ratio) predict cognitive abilities at 56 (±1 month) months of age among Finnish children born at term and free of any major developmental disabilities/impairments (WHO criteria). **Methods**. Participants were derived from representative regional cohort of 2193 Finnish neonates born in 1985-1986. The participants of the current study were 973 children who were born at term, had no inborn or later developed major developmental impairments/disabilities and had cognitive test scores available at 56 months of age. Gestational age and body size measures were derived from birth records. At 56 months' follow-up four pediatricians performed cognitive ability tests to children measuring general reasoning (CMM), verbal competence (AWST), language comprehension (LVST-a and -c), and visual-motor integration and functioning (VMI). Covariates included gestational age (in analyses of body size), child's gender, multiple pregnancy, neonatal neurological optimality score, and maternal age, education, body mass index before pregnancy and smoking during pregnancy, and whether the child had been hospitalized or not during the first 10 days after the birth. Control measures were derived from the hospital records and via maternal interviews.

Results. After adjustments for the covariates, the results showed that weight, length and head circumference at birth were significantly correlated with general reasoning (CMM) (β's = .09 to .11, p's < .02), with verbal competence (AWST) (β's = .09 to .15, p's < .02), with visual-motor integration and functioning (VMI) (β's = .10 to .13, p's < .006) and with langue comprehension (LSVT-a, performance part, and length only LSVT-c, description part) (β's = .08 to .10, p's < .03) at 56 months of age. Gestational age was significantly associated with visual-motor integration and functioning (VMI) (β = .08, p = .02) and with langue comprehension (LSVT-c, description part) (β = .07, p = .03). Gender of the child did not moderate any of the associations. **Conclusions**. Results indicate that also among children born at term gestational age, weight, length and head circumference at birth are significantly associated with cognitive abilities at 56 months of age. These results highlight that cognitive abilities in early childhood have prenatal origins among children born at term and are in concordance with the fetal programming hypothesis.

P1-071

Postnatal Bacterial Infection as a Predictor of Post Traumatic Stress Disorder: Physiological and Behavioural Correlates in the Rodent Brendon Knott, Frederick, R. Walker & Deborah M. Hodgson; Laboratory of Neuroimmunology, School of Behavioural Sciences, University of Newcastle, Callaghan, 2308, NSW, Australia

Exposure to early life stress in humans has been shown to enhance susceptibility to the onset of a variety of psychiatric disorders during adulthood, including depression and post traumatic stress disorder (PTSD). Dysregulation of the hypothalamo-pituitary-adrenal (HPA) axis following early life stress is well documented in the rodent literature, and, in humans, as a contributing factor in this process. A common stressor during early life is exposure to bacteria. Exposure of rodents in the first week of life to bacterial endotoxin alters the development of the HPA axis such that when these same animals are exposed to stress in adulthood they are hyper-responsive. The current study is the first to explore the modulatory effect of postnatal bacterial exposure on susceptibility to psychopathology using a rodent model of PTSD. The PTSD model utilised predicts that a subpopulation of rodents exposed to stress as adults will develop enduring nonhabituation of the acoustic startle response (ASR). The question of interest in this study was whether early life exposure to a physiological stressor, immune activation, would alter this predisposition. Long Evans neonatal rats were exposure to bacterial endotoxin (50 μg/kg *Salmonella enteritidis,i.p.*), or an equivalent volume of saline on days 3 and 5 of life. In adulthood, anxiety-like behaviours were measured using standard behavioural tests including the elevated plus maze (EPM), the hole board, and the open field. HPA responsiveness was assessed according to serum corticosterone levels obtained from blood samples taken at 30, 60 and 90 min after 30 minutes of immobilisation stress. ASR habituation was assessed in adulthood on day 1, 3, 5 and 7 following two immobilisation stress sessions (each 30 minutes) and a 30 minute social isolation session in a novel environment room. To assess ASR habituation each animal was placed into a startle apparatus and exposed to 150 startle bursts. The mean startle amplitude for the first 10 trials and last 10 trials were taken for analysis. The results indicate that adult rats exposed to endotoxin as neonates demonstrated significantly higher levels of circulating corticosterone than saline treated controls in response to 30 minutes of immobilisation stress at 60 and 90 minutes post stress ($p<.05$), indicating hyper-responsivity, and impaired dampening of the HPA response. Locomotor and exploratory behaviours on the hole board test were significantly higher in endotoxin treated neonates than saline controls ($p<0.05$), though no differences between the groups were observed on the EPM or open field tests. Lastly, endotoxin challenged animals exhibited higher mean ASR amplitudes, indicative of increased anxiety post stress than animals in any other treatment group, at 1, 3, 5 and 7 days following adult stress exposure. This difference was observable up to 7 days following adult stress exposure. This indicates that exposure to an immune challenge in early life elicits enduring dysregulation of the physiological and behavioural rodent stress response. These factors may act as a marker for enhanced susceptibility to PTSD and other forms of psychopathology. These findings have implications for the further investigation of mechanisms mediating psychopathological vulnerability following early life stress.

P1-072

Maternal Smoking During Pregnancy and Children's Physical Aggression: Results of a Twin Study Stephan C.J. Huijbregts, Jim Stevenson, Michel Boivin, Jean R. Séguin, Richard E. Tremblay; *School of Psychology, University of Southampton, Southampton, SO17 1BJ United Kingdom. Department of Psychology, Université Laval, Québec City, Canada. Departments of Pediatrics, Psychiatry and Psychology, Université de Montréal, Montréal, Canada.*

Background Maternal smoking during pregnancy is associated with children's physical aggression. Heritability has been estimated to account for 50% to 70% of the variance in children's physical aggression. A number of recent twin studies (e.g. Maughan et al, 2004. *Arch Gen Psychiatry, 61*. 836-43) questioned whether there is a direct (causal) path from mother's smoking to children's antisocial behaviour and suggested the association could be accounted for by parental (history of) antisocial behaviour, either through genetic transmission of antisocial traits or through its influence on several environmental factors such as harsh parenting and socioeconomic adversity. We reinvestigated the relationship between pregnancy smoking and children's physical aggression in a twin sample using similar variables and data analytic techniques as Maughan and colleagues. **Methods** Mother ratings of physical aggression in 286 same sex twin pairs at age 48 months were collected using the preschool behavior scale from the Canadian National Longitudinal Study of Children

and Youth. Cigarette smoking during pregnancy was subdivided into 4 categories (1 = no smoking; 2 = 1-10/day; 3 = 11-20/day; 4 = >20/day). Antisocial behavior in mothers and fathers was assessed with items from the NIMH-Diagnostic Interview Schedule. Symptoms associated with maternal depression were assessed with the CES-D Scale. A composite score of maternal education and family income reflected socioeconomic status. In order to analyze whether pregnancy smoking predicted children's physical aggression over and above the influence of gender, heritability and environmental factors a multiple regression analysis was performed, using an extension of the DeFries and Fulker method, which predicts twin aggression from that of its co-twin. The statistical package used for the analyses was STATA. **Results** In a model with gender, heritability of physical aggression, and environmental factors [$F(8,286) = 41.5$, $p < .001$, $R^2 = .35$], maternal smoking during pregnancy remained a significant predictor of children's physical aggression (ß = .08 (95% CI 0.03 - 0.14), p = .002). The heritability estimate (ß = .66 (95% CI 0.54 - 0.78), p < .001) was the strongest predictor of physical aggression, while gender (ß = -0.09, p = .002) and father's history of antisocial behaviour (ß = .06, p = .006) were also significant predictors. There was no change in the amount of variance uniquely associated with pregnancy smoking when parental antisocial behaviour and (other) environmental factors were controlled for (ß = .17 (2.9%)), but the amount of unique variation significantly decreased when heritability of physical aggression was taken into account (ß = .08 (0.6%)). **Conclusions** In contrast with the results by Maughan and colleagues, maternal smoking during pregnancy had a small but significant influence on offspring physical aggression even after control for heritability of physical aggression. Heritability of physical aggression in our data set mainly involved direct genetic transmission of antisocial traits. This suggests that in utero exposure to nicotine and genes associated with physical aggression independently affect behaviour. However this may be through a common impact on catecholaminergic and serotonergic neurotransmission.

P1-073

Early Life Effects on Cerebral Hemispheric Activation: Evidence From a Study of Tympanic Membrane Temperature Asymmetry Alexander Jones, Clive Osmond, Keith M. Godfrey and David I. W. Phillips; *MRC Epidemiology Resource Centre, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK.*

Background: The tympanic membrane is an indirect, but reliable, site from which to measure intracranial temperature. Neurosurgical studies show that cortical temperature is highly correlated with tympanic membrane temperature in the ipsilateral ear. Recent studies have suggested that tympanic membrane temperature asymmetry may be a phenotypic marker of vulnerability to negative emotionality in children. Because increasing evidence suggests that adverse fetal environments are associated with negative affect or depression, we have evaluated whether fetal growth is linked with this measurement of hemispheric activation. **Aim**: We investigated whether size at birth correlates with tympanic membrane temperature asymmetry. **Methods**: We have carried out a cross-sectional study of 68 boys and 72 girls (aged 7-9 years) who have been followed since 12 weeks of gestation when their mothers took part in a study of healthy children born in Southampton, United Kingdom. Whilst the children took part in a study of the impact of prenatal environment on physiological responses to psychological stress, we measured bilateral tympanic membrane temperature using a pair of infrared tympanic thermometers (Braun Thermoscan Pro 3000) simultaneously on four occasions (25, 70, 200 & 230 minutes after arrival). Each pair of measurements was taken twice with the measurers and their thermometers swapping ears to account for small differences in thermometer calibration and measurement technique. Side of first measurement and choice of thermometer for each user on each occasion were randomized by computer. Thermometer calibration was regularly assessed using a Diatek 9600 blackbody device (error < 0.2ºC). Analysis was performed using repeated measures linear regression. **Results**: The mean difference in tympanic membrane temperature between left and right ears was normally distributed about zero with a range of -0.7 to 0.9ºC. Although this difference was unrelated to gender or current body size, left handedness predicted a 0.15ºC warmer right tympanic membrane and low birthweight was associated with a warmer left tympanic membrane ($r = -0.2$, $P = 0.02$). This association between size at birth and tympanic membrane temperature was more marked in low birthweight individuals with a relatively larger placenta ($r = -0.24$, $P < 0.001$). Due to their influence on tympanic membrane asymmetry, mean external temperature and handedness were accounted for in our analysis. **Conclusion**: This is the first study to demonstrate that altered fetal growth is associated with tympanic membrane temperature asymmetry. As there is increasing evidence that tympanic membrane temperature asymmetry is associated with intracranial differences in blood flow and electroencephalogram activity, our findings suggest that low birthweight is characterized by altered laterality of brain function.

P1-074

Fetal Programming of Temperamental Negative Affectivity Among Children Born Healthy and at Term *Anu-Katriina Pesonen, Katri Räikkönen, Eero Kajantie, Kati Heinonen, Timo E Strandberg, Anna-Liisa Järvenpää, Department of Psychology, University of Helsinki, P.O. Box 9, 00014 University of Helsinki, Finland. anukatriina.pesonen@helsinki.fi*

Background and methods: The fetal programming hypothesis suggests that an adverse *in utero* environment, indicated by small body size at birth, has life-long effects on different physiological systems, such as the hypothalamic-pituitary-adrenal (HPA) axis. We explored whether fetal growth was associated with HPA axis-related temperamental outcomes (negative affectivity scales, the CBQ) among 416 5.5-year-old-children born healthy at term (gestational weeks 37-42). **Results:** In line with the hypotheses, thinness at birth (measured by ponderal index, kg/m^3) was related to increased negative affectivity and its subscales, anger- discomfort- and sadness-proneness in childhood. As assumed, a lower birth weight was related to increased negative affectivity, but only among children born at early-term weeks of gestation (37-39), in accordance with the proposed link between the fetal glucocorticoid environment and child negative affectivity. **Conclusions:** The results support the idea of the malleability of the biological basis of temperament. Significantly, they also indicate that mechanisms behind associations found operate within the normal range of term birth.

P1-075

Depressive Symptoms and Self-reported Health in Middle Age after Intrauterine Undernutrition during the Dutch Famine (1944-1945) Frank H. Pierik, Karin M. van der Pal-de Bruin, GHW (Erik) Verrips, Patricia A Zybert, LH Lumey. *Dept of Reproductive Health, TNO Quality of Life, Leiden, The Netherlands; Mailman School of Public Health, Columbia University, New York, NY, USA.*

Background: Few studies in humans have assessed the effect of maternal nutrition during pregnancy on measures of mental and physical health in adult offspring. We used the circumstances of the Dutch Famine of 1944-45, during which official rations were <900 kcal/day for 24 weeks, to assess whether generalized reductions of maternal food intakes at specified stages of pregnancy were related to depressive symptoms and self-reported health in adulthood. **Methods:** Famine effects were studied two series of subjects: (1) exposed individuals born in one of three institutions in western Holland between January 1945 and March 1946, whose mothers were all exposed to the famine during or immediately preceding pregnancy, and (2) unexposed individuals born in the same three institutions during 1943 or 1947, whose mothers had no famine exposure during this pregnancy (n=176). We defined four (partially overlapping) windows of gestational exposure (gestational weeks (GW) 1-10 (n=85); 11-20 (n=140); 21-30 (n=157); and 31 through delivery (n=142)) based on exposure to a ration <900 kcal/day during the whole 10-week interval. All study subjects were examined between 2003 and 2005 when they were on average 59 years old. Depressive symptoms were assessed by the Center for Epidemiologic Studies Depression scale (CES-D). Self-reported health status - expressed as the mental component score (MCS) and physical component score (PCS) - was measured with the Short Form 36 questionnaire (SF-36). Both the CES-D and SF-36 questionnaires are widely used and accepted, have been validated for use in the Dutch population, and were completed by study subjects during a medical examination in a central location. Data on health history in several health domains such as cardiovascular disease, diabetes, obesity, and blood pressure were also collected. **Results:** Compared to the unexposed controls with an average depressive symptom score of 10.9 (sd 7.3), all exposed groups had an elevated depressive symptom score. This was statistically significant for GW 1-10 (13.6; sd 8.0), GW 11-20 (13.5; sd 7.9), GW 21-30 (13.1; sd 7.7), and for the full period of GW 1-birth (12.3; sd 7.6; ANOVA; $p<0.05$). Compared to unexposed controls, famine exposure during gestation (pooled exposure periods) was also associated with a depressed mental component score (53.7 vs 51.9, respectively; ANOVA; $p<0.05$), but for separate exposure periods the consistently lower MCS was not statistically significant. There was no interaction with gender and the effects persisted after control for self-reported history of myocardial infarction, heart surgery, stroke, high-blood pressure, high cholesterol, and diabetes. The MCS and the CES-D scores were significantly correlated (Spearman's correlation coefficient: 0.7; $p<0.05$). No significant effect of famine exposure on perceived physical health was observed. **Conclusions:** Famine exposure during gestation was associated with a lower perceived mental health and a higher level of depressive symptoms, irrespective of specific chronic conditions. These findings need further exploration.

P1-076

Birthweight Influences On Adult Human Motor Function and Cortical Excitability Julia Pitcher [1], Alexandra Robertson [1], Timothy Miles [3], Richard Cockington [4] & Vivienne Moore [2]. Departments of Obstetrics & Gynaecology [1], Public Health [2], and Discipline of Physiology [3], The University of Adelaide; Department of Child Development and Rehabilitation [4], The Women's & Children's Hospital, Adelaide, AUSTRALIA 5005.

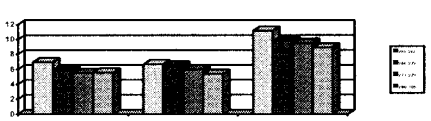
Background: Parents and teachers of children born small for gestational age (SGA) often report these children being "clumsy" and having poor motor skills. However, it is unknown if these motor deficits persist into adulthood, their extent and impact on neuromotor function for the individual, and what underlying physiological determinants of that function have been impaired. We examined if adults born SGA exhibit impaired motor control, and if this is due to altered corticospinal tract function, the major descending motor pathway in humans. **Methods**: We present preliminary data from a cohort of 856 28-year-old members of the Adelaide Family Heart Study whose birth characteristics were recorded in detail. Birthweights ranged from 1470g-4710g and gestation was >36 weeks. Corticospinal stimulus-response curves for a right hand muscle were constructed using transcranial magnetic brain stimulation and surface electromyography. Motor thresholds and cortical silent periods were obtained for both hands. Nerve conduction velocity was also recorded. Motor skill tests were compared with the corticospinal data, including handgrip strength, finger tapping speed and a Purdue pegboard test of dexterity. Handedness was established using the Edinburgh Handedness Inventory. **Results**: Lower birthweight was associated with a larger interhemispheric difference in threshold motor cortex excitability (IHTD) ($r^2=0.47$, $P = 0.003$, N=37) with the right hemisphere (left hand) threshold (LHT) being higher than the left (right hand) (RHT). This birthweight-associated difference

was more prominent in females than in males. Functionally, however, a larger IHTD only affected males and was associated with a higher (i.e. more right handed) laterality quotient (r^2=0.76, P = 0.001, N=16), a lower LHT (r^2= -0.55, P = 0.03, N=16) and poorer left hand pegboard (r^2= -0.57, P = 0.02, N=16) and tapping (r^2=-0.53, P = 0.03, N=16) scores. Conversely, low birthweight (r^2=-0.48, P = 0.05, N=17) and shorter gestation (r^2=-0.51, P = 0.04, N=17) in males were associated with faster right hand tapping speeds. In females, a larger IHTD was also associated with a lower LHT (r^2= -0.64, P = 0.002, N=21) and a steeper cortical curve slope (r^2= -0.59, P = 0.01, N=21), but not with any motor skill outcomes. Shorter gestation females had reduced ulnar nerve conduction velocity (r^2= 0.58, P = 0.007, N=20). Overall, lower birthweight subjects had steeper cortical curve slopes (r^2=0.35, P = 0.04, N=35) and a trend for a shorter silent period in the right motor cortex, particularly in females. Lower birthweight males but not females had reduced handgrip strength in both right (r^2=0.51, P = 0.03, N=37) and left hands (r^2=0.55, P = 0.03, N=17), but this was not associated with any corticospinal measures. **Conclusions**: Birthweight influences corticospinal and motor function outcomes and differently in males and females. Both exhibit increased asymmetry in interhemispheric excitability of the motor cortices, but this appears to affect simple motor task performance only in males. The increased curve slopes suggest increased excitability in the left hemisphere, while the shorter silent periods in the right cortex suggest reduced inhibition of the left hand cortical representation. The full functional implications of this are yet to be elucidated, but greater cortical excitability asymmetry in low birthweight males surprisingly seems to confer a higher likelihood of being more right than left handed, and superior right hand fine motor skills at the expense of left hand skills. In females, the picture is less clear with more distinct corticospinal changes, but no concomitant alteration in simple motor skill performance. Reduced strength in low birthweight males appears not to be cortical in origin.

P1-077

Born Earlier and Smaller: Implications for Depression in Later Life among Men and Women Born at Term in Helsinki 1934-44. Katri Räikkönen, Anu-Katriina Pesonen, Eero Kajantie, Kati Heinonen, Tom Forsen, David I.W. Phillips, Clive Osmond, David J.P. Barker and Johan G. Eriksson Department of Psychology, 00014 University of Helsinki, Helsinki, Finland, National Public Health Institute, 00300 Helsinki, Finland, MRC Epidemiology Resource Centre, University of Southampton, Southampton SO16 6YD, UK, DOHaD Centre, University of Southampton, Southampton SO16 6YD, UK.

Background: Against a widespread belief, epidemiological evidence linking body size at birth with depression later in life is scanty and the results are mixed. **Objective:** To test the hypothesis that shorter gestational duration and smaller body size at birth predict depression in late life. **Design:** Longitudinal study of 1510 members of the Helsinki Birth Cohort born1934-44 and followed to age 59.7 - 70.7 years. ***Methods:*** 1510 cohort members (668 men and 842 women) born at term (259 – 309 days' gestation) completed the Beck Depression Inventory (BDI), and the Center for Epidemiological Studies Depression Scale (CES-D) on two separate occasions on average 1.88 (SD = 0.73) years apart, in 2001-2003 (BDI) and in 2004 (BDI and CES-D). Data abstracted from birth records included gestational duration, weight, length, and head circumference. **Results:** After adjusting for gender, the most robust early determinant of depression was shorter gestational duration (Figure 1). The risk for fulfilling the BDI criteria of mild to severe depression simultaneously at the two testing sessions over time increased with shorter gestational duration (OR, 1.35 per week; 95%CI, 1.15 – 1.58). Similarly, the risk for fulfilling the BDI and the CES-D criteria of mild to severe depression contemporaneously in the 2004 testing session increased with shorter gestational duration (OR, 1.32 per week; 95%CI, 1.13 – 1.53). Weight, length and head circumference at birth (adjusting for gender and gestational duration) showed no significant associations with depression. However, those weighing < 2.5 kg compared to those weighing more (ß's = .06 to .08, p's < .03 to .002), and those being < 48 cm in length at birth compared to those being taller (ß's = .05 to .08, p's < .05 to .002) scored significantly higher on depression in late life. The associations did not vary significantly by gender, nor were they explained by age at the time of measuring depression, years of education, nor by adult body size.



BDI 2001-2003	BDI 2004	CES-D 2004
F=3.97, p<.011	F=4.01, p<.007	F=3.95, p<.008
p<01 for trend	p<.018 for trend	P<.014 for trend

Figure 1. Depressive symptoms in later life according to gestational duration (adjusted for gender).

Conclusions: Altered hypothalamic-pituitary-adrenal axis activity is among the most consistently demonstrated biological abnormalities in depression, is involved in the timing of parturition, and characterizes individuals born earlier and smaller. Other associates may include growth hormones, and neurotransmitters. Early antenatal programming of these systems may explain the findings.

Gestational duration (days):

P1-078

Post-natal Growth and Cognitive Development in a Population-based Birth Cohort. Marcus Richards[1], Stephani Hatch[2], Rebecca Hardy[1], Diana Kuh[1], Ezra Susser[2] and Michael Wadsworth[1]. [1]*MRC National Survey of Health and development, University college London, London, UK.* [2]*Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, USA.*

Background: There is now a consensus that fetal growth, represented by birth weight, is positively associated with cognitive development in the general population, independently of socio-economic status (SES). However, less is known about the effect of post-natal growth on cognitive development. While the growth acceleration hypothesis proposes that fetal growth restriction followed by enhanced infant growth increases risk of cardiovascular disease, there is some evidence that post-natal growth in those born small for gestational age benefits cognitive development. **Methods**: Based on data from the British 1946 birth cohort we used multiple linear regression models to test the effect of height and weight at ages 2, 4 and 7 years on general cognitive ability at 8 years, controlling for birth weight, parental SES, and birth order. We also tested the effect of height and weight change between birth to 2, 2 to 4, and 4 to 7 years, then investigated interactions between birth weight and measures of post-natal size. **Results**: After controlling for birth weight, father's occupation, mother's education, mother's age at birth, and birth order, we found that height growth between 2 and 4 years was positively associated with cognitive ability at 8 years, independently of weight gain, with an effect of similar magnitude to that of birth weight. This height growth benefited mostly those of relatively low birth weight. **Conclusions**: Growth in early childhood is positively associated with cognitive development independently of fetal growth, and this may be particularly true in those who were born small. The consistency of these results will be examined through careful comparison with the USA National Collaborative Perinatal Project.

Placenta

P1-079

Differential Expression of the Prostaglandin Enzyme COX-2 is Altered in Late Gestation Sheep Placentomes by Administration of Maternal Betamethasone Thorsten Braun[1,2], Kristin Connor[1], Shaofu Li[4], Timothy Moss[3,4], John P. Newnham[3,4], John R.G. Challis[1] and Deborah M. Sloboda[3,4]; [1] Department of Physiology and Obstetrics and Gynecology, University of Toronto, Toronto, Canada; [2] Heinrich Heine University Düsseldorf, Department of Obstetrics and Gynecology, Düsseldorf, Germany; [3] School of Womens' and Infant's Health, The University of Western Australia, Perth, Australia; [4] Women and Infants Research Foundation, King Edward Memorial Hospital, Perth, Australia

Objective: Maternal betamethasone administration in sheep results in reduced fetal weight and may be mediated by effects on the placenta. We have shown a reduction in placental weight and alterations in placentome subtype distribution in sheep after betamethasone exposure. It is unknown whether maternal betamethasone administration alters placental enzyme expression. Therefore we investigated the expression of COX-2 enzyme in different placentome subtypes after maternal betamethasone administration. **Methods:** Pregnant ewes were randomised to control or betamethasone (0,5 mg/kg ewe weight, n=11) groups and injected with saline or betamethasone at 104, 111 and 118 days of gestation (d). Animals were sacrificed prior to (d75, d101) and after (d121, d146) betamethasone. Fetal weights were recorded; placentomes were classified according to their gross morphology into A, B, C, D subtypes and one of each subtype was collected. Western Blot was used to measure placental COX-2 expression. **Results:** Placental COX-2 protein expression in control animals significantly increased over gestation [p<0,05] with highest levels observed at d146. There was no different COX-2 expression between the placentome subtypes in control animals at d75, d101, d121 and d146. At d121 overall placental COX-2 protein expression tended to be higher in the treated group but this difference was not statistically significant. In betamethasone treated animals at d146, C subtype placentomes expressed significantly higher levels of COX-2 protein compared with A subtypes [p<0.05]. This differential expression was not apparent in placentomes from control animals. **Conclusions:** Maternal betamethasone administration resulted in the differential expression of COX-2 protein expression in sheep placentomes late in gestation. Although it is unknown whether these alterations translate into functional changes, it appears that prenatal exposure to betamethasone may contribute to differential changes in placentome subtype protein expression and placental development that may contribute to downstream effects on the fetus.

P1-080

Associations between Placental Pathology, Race, and Socio-economic status in the US, 1959-1966: Results of the National Collaborative Perinatal Project Tarayn A. GRIZZARD, BS, BA,[1] Carrie SALAFIA, MD, MS,[2] Matthew Gillman, MD, SM,[1] Stephen BUKA, ScD,[3] and Janet RICH-EDWARDS, ScD, MPH[1] [1]Department of Ambulatory Care & Prevention, Harvard Medical School, Boston, MA, 02215 [2]Department of Epidemiology, Mailman School of Public Health, Columbia University [3]Department of Society, Human Development, and Health, Harvard School of Public Health

Background Black and low socio-economic status (SES) women have higher rates of preterm birth (PTB) and (FGR), even after controlling for known risk factors such as smoking. Studies have shown some placental histopathologic findings to be associated with PTB and FGR, suggesting a possible intermediate pathway. **Objectives** We

determined if placental histopathology consistent histologic chorioamnionitis and maternal vasculopathy is associated with PTB and/or FGR, and whether it accounts for the higher rates of PTB and FGR among black and low-SES women. **Study Design** We analyzed data from the Boston cohort (n=13,601) of the National Collaborative Perinatal Project (NCPP). We included live-born singleton infants born from 20 to 44 weeks' gestation born to white or black mothers who had complete placental pathologic exam records (n=11,337). We used logistic and linear regression analysis to determine associations between race and SES and placental histopathology, and between placental histopathology and PTB and FGR. **Results** We detected histological chorioamnionitis in 2965 (26%) and maternal vasculopathy in 2546 (22%) of Boston study participants. Women with moderate or severe histological chorioamnionitis had a small (14%, OR 1.14, 95%CI 0.90, 1.45) increased risk of PTB. Mild histological chorioamnionitis was not associated with PTB. Neither mild nor moderate-severe histological chorioamnionitis was associated with FGR. Maternal vasculopathy was not associated with either PTB or FGR. Combined histological chorioamnionitis and maternal vasculopathy were not associated with PTB or FGR. **Conclusion** Histologic chorioamnionitis and maternal vasculopathy as we defined them within the NCPP were not associated with race or SES, or with PTB or FGR. Histologic chorioamnionitis and maternal vasculopathy did not account for the associations of black race or low SES with PTB or FGR.

P1-081

Early Steps in Fetal Programming: Global Nutrition Restriction Augments Small Heat Shock Protein-27 (HSP-27) in Rat Placenta Jayaraman Lakshmanan and Michael G. Ross, *Department of Obstetrics and Gynecology, David Geffen School of Medicine at UCLA and LABioMed at Harbor-UCLA Medical Center, Torrance, CA 90502, USA*

Background: Inadequate maternal nutrition is a widely used experimental paradigm in animal models to elucidate the developmental origin of adult diseases. Maternal nutrient restriction has consistently demonstrated the association of an adverse intrauterine nutritional environment and programming of offspring hypertension, diabetes, obesity and other chronic diseases. However, little attention has been focused on the underlying molecular mechanism(s), which are relevant to early diagnosis and/or prevention of programmed adult onset diseases. Since the placenta plays an essential role in nutrition exchange between mother and fetus, we hypothesized that placental cells should respond to nutrition restriction by activating stress proteins or protein chaperones that protect vital protein structures and functions. In the present study we tested our hypothesis by examining the expression of placental small heat shock protein-27 (HSP-27) in response to maternal nutrient restriction. **Methods**: Time-dated pregnant Sprague-Dawley rats were subjected to 50% food restriction from day 10 to 15 of gestation and sacrificed on day 16. Control rats were provided with food and water ad libitum. Placental tissues were dissected, fixed in 4% paraformaldehyde and paraffin embedded. Sections were cut and immunostained with rabbit antibodies to mouse recombinant HSP-27 by avidin-biotin-immunoperoxidase complex method. Sections were counterstained with hematoxylin and examined under a microscope. **Results**: In placenta of food-restricted rats, the HSP-27 antibodies strongly immunostained the cytoplasm of giant trophoblast cells at the junctional zone and trophoblast cells in the labyrinth. Placentas of maternal rats fed ad libitum demonstrated very weak HSP-27 immunostaining both in junctional and labyrinth zones. **Conclusion**: The abundant presence of HSP-27 in the placenta of food restricted mothers suggests a natural cytoprotective mechanism which may maintain the cellular organization and functions of macromolecules. We speculate that forced overexpression of HSP-27 may protect the fetus from programming of adult stage diseases. Cytoprotective agents should provide a new avenue to prevent the developmental origin of adult diseases.

P1-082

The Association of Prenatal Vitamin C Status with Placental Apoptosis and Oxidative Stress in Normal Pregnancy Hwayoung Lee[1,4], Young-mo Ahn[1], Young Ju Kim[3,4], Hyesook Park[2,4], Bohyun Park[2]; [1]*Department of Anatomy and* [2]*Preventive Medicine, Ewha Womans University Medical College, Seoul, Korea, Republic of;* [3]*Department of Obstetrics & Gynecology, Ewha Womans University Hospital, Seoul, Korea, Republic of;* [4]*Ewha Medical Research Center, Seoul, Korea, Republic of*

Background: The adequate amounts of vitamins and minerals during development are essential for both the immediate and long-term well-being of the embryo, fetus and neonate. Antioxidant vitamins are of particular importance during pregnancy. Their deficiencies occurring during pregnancy and placental oxidant-antioxidant imbalance may perturb the development of feto-placental unit or offspring in the absence of clinical signs of deficiency in the mother. The aim of this study was to determine the possible association of prenatal status of plasma vitamin C with the oxidative stress and apoptotic activity in normal term human placentas. **Methods**: We evaluated the placental lectin-like oxidized low-density lipoprotein receptor-1(LOX-1) contents and trophoblast apoptotic index from 80 normal full-term human pregnancies using immunohistochemistry and western blot analysis, and then investigated their correlation with prenatal vitamin C status. **Results & Conclusions**: We showed that the prenatal vitamin C status might contribute to the placental status of oxidative stress and trophoblast apoptotic activity in normal term human pregnancy. We confirmed that the trophoblast expression for endothelial scavenger receptor LOX-1 was downregulated and the apoptotic activity in syncytiotrophoblastic cells was significantly decreased in term placentas from normal pregnancies with high level of prenatal vitamin C compared to those from pregnancies with low prenatal vitamin C in vivo. Our results may indicate that prenatal status of antioxidant vitamin C can influence on the fetal or neonatal potential outcomes through its effects on intra-uterine environment in normal term pregnancy. Therefore, this study suggests that the status of prenatal vitamin C can affect fetal environment through alterations in placental function, and consequently influence on long-term well-being of neonate. This study was funded by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (03-PJ1-PG10-21900-0001)

P1-083

Lactate Produced by the Isolated Perfused Human Placental Cotyledon is Predominantly Released into the Maternal Circulation RM Lewis[1], P Brownbill[2], CP Sibley[2], IT Cameron[1], MA Hanson[1]. [1]DOHaD Centre University of Southampton, UK. [2] The Division of Human Development, The Medical School, University of Manchester, UK.

Introduction: Placental nutrient transport is a major determinant of fetal growth and is likely to be an important mediator of developmental phenotypic induction. The sheep placenta converts large amounts of glucose to lactate which is released into both the umbilical and uterine circulations. Lactate secreted into the umbilical circulation is a major energy source for the sheep fetus. To study this in the human we perfused isolated human placental cotyledons with glucose and measured maternal and fetal glucose uptake and lactate release. **Methods**: The fetal and maternal arterial circulations of human placental cotyledons were perfused at 6 and 14 ml/min respectively in an open circuit with a modified Earle's buffered saline containing 5.5mM D-glucose, 35g/l dextran, 0.1% BSA and 25000IU/l Heparin at 37°C and gassed with 95% O_2/5% CO_2. Only placentas with a fetal venous recovery = 95% were used. Glucose uptake and lactate levels were determined, using standard enzymatic assays, in fetal and maternal venous perfusate collected between 60 and 150 minutes from the start of fetal perfusion. **Results**: Placental lactate release into maternal venous effluent was 8 times higher than that into fetal venous effluent (8.2 ± 1.1 nmol/min/g *vs.* 1.1 ± 0.4 nmol/min/g, P<0.001, n=5). Glucose uptake by the placenta was greater from the maternal circulation than from the fetal circulation (229 ± 81 nmol/min/g *vs.* 73 ± 48 nmol/min/g, P<0.05, n=5). **Conclusions:** These data indicate that, unlike in the sheep, in the human placenta lactate efflux occurs primarily in the placental to maternal direction. This suggests that placentally-derived lactate is unlikely to be a major energy source in the human fetus and that human fetuses may be more reliant on transport of alternative energy substrates, such as glucose, fatty acids or amino acids. The study provides further evidence for differences in placental metabolism and function between human and sheep.

P1-084

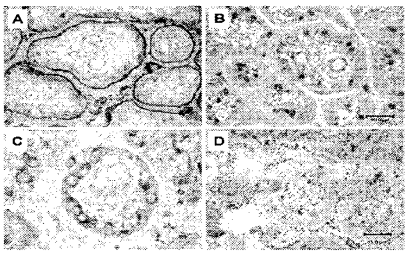
Maternal Baboon 30% Global Nutrient Restriction (MNR) from 30 to 90 Days of Gestation (dG) Alters Placental Trophoblast Leptin and Leptin Long Form Receptor (Ob-R) Distribution Li C., Schlabritz-Loutsevitch N., Hubbard, G.[2],Nathanielsz P.W., McDonald T.J., Center for Pregnancy and Newborn Research, Dept. of Ob/Gyn, Univ. of Texas Health Science Ctr., San Antonio, TX and [2]Southwest Foundation for Biomedical Research, San Antonio, TX

Introduction: In humans, leptin is: 1) produced by placental trophoblast, 2) secreted into both maternal and fetal circulation, 3) increased in diabetic pregnancy and pre-eclampsia and 4) reduced in maternal and fetal plasma in intrauterine growth restriction (IUGR; Placenta 23:103). The present study examined MNR effects on baboon placental leptin and Ob-R protein expression at 90 dG in cytotrophoblast (CT) in comparison to syncytiotrophoblast (ST). **Methods:** MNR baboons were fed 30% less than *ad libitum* fed controls (*ALC*) from 30 dG to tissue collection at 90 dG. Standard immunocytochemistry was done using leptin and long form Ob-R antibodies (1:50 final dilution). Field fraction was measured by image analysis. **Results:** *ALC* body weight did not change (13.4 ± 0.6 vs 13.61 ± 0.4 kg; n = 8), while MNR weight decreased (13.0 ± 0.2 vs 12.2 ± 0.3 kg; n = 6; p < 0.05) from 30 to 90 dG. Fetal (*ALC*-100.9 ± 3.4 vs MNR 95.4 ± 3.3 g) and placental (*ALC* 70.4 ± 5.1 vs MNR 62.9 ± 1.5) weights were not different at 90 dG. Placental leptin immunoreactivty was primarily located in *ALC* ST as a dense band on the maternal-blood side while MNR placentas exhibited less intense leptin immunogenicity primarily in CT (Fig. 1A & B). Ob-R in was found primarily in *ALC* cytoplasm of CT while MNR placenta showed membrane bound Ob-R in both ST and CT (Table 1).

	CT Leptin	ST Leptin	CT Ob-R	ST Ob-R
ALC	4.97 ± 2.3	43.4 ± 6.8*	57.1 ± 6.3*	20.1 ± 7
MNR	40.6 ± 5.5*	3.61 ± 1.9	25.5 ± 7.1	28.6 ± 5.6

Table 1. Fraction immunostained for leptin or leptin receptor (Ob-R) in CT or ST from *ALC* and MNR baboons at 90 dG. * (p< 0.05).

Figure 1. Placental leptin (A & B) and Ob-R (C & D) in *ALC* (A & C) and MNR (B & D) placentas. Note the location of: 1) leptin in ST of *ALC* vs in CT of MNR and 2) Ob-R in CT cytoplasm of *ALC* vs membrane bound in CT and ST of MNR placentas.



Conclusions: Given that in normal development, villous CT fuse to form the ST, the changes in baboon placental leptin and Ob-R distribution with nutrient restriction suggest a delay in maturation of leptin and Ob-R expression in MNR placentas. The importance of such alterations may be related to changes with pathologies of nutrient supply such as the reduced plasma leptin in human IUGR fetuses.

P1-085

P-glycoprotein Limits Dexamethasone-induced Activation of the Glucocorticoid Receptor in Placental BeWo Cells: Implications for the Placental Glucocorticoid Barrier Peter J Mark and Brendan J Waddell; *School of Anatomy and Human Biology, The University of Western Australia, Crawley, Western Australia, 6009 Australia.*

Introduction. Fetal glucocorticoid exposure is a key determinant of fetal growth and programming of adult-onset diseases including hypertension, type II diabetes and obesity. Glucocorticoids potently inhibit fetal and placental growth via activation of the glucocorticoid receptor (GR). Placental and fetal glucocorticoid exposure is minimised by the "placental glucocorticoid barrier" which consists primarily of placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) mediated conversion of maternal glucocorticoids to inactive metabolites. Recent studies in the rodent brain show that P-glycoprotein (P-gp) is also an important physiological regulator of glucocorticoid access to the GR in target cells, preventing glucocorticoids from crossing the blood brain barrier by exporting them back into circulation against a concentration gradient. Therefore, we hypothesised that placental P-gp may be ideally located at the syncytial brush border to prevent maternal glucocorticoids traversing the placenta to access the fetus, and thereby augments the placental glucocorticoid barrier. We have used the human placental choriocarcinoma cell line BeWo, and BeWoMDR, a daughter cell line virally-transduced with P-gp [1], to assess whether placental P-gp regulates access of dexamethasone to the GR. **Methods**. For studies investigating syncytialization, BeWo and BeWoMDR cells were dosed with either vehicle or 20 μM forskolin for 72 hours prior to quantitation of P-gp mRNA by realtime RT-PCR. GR activation was determined by transfection of cells with 500 ng of the GR expression plasmid pRSVhGR, 500 ng of reporter plasmid pGRE Luciferase and 50 ng of internal control plasmid, pTK Renilla. Following transfection, cells were dosed with either vehicle or appropriate doses of dexamethasone (10^{-10} M to 10^{-6} M) or the anti-glucocorticoid, RU486 (5 μM), for 24 hours prior to harvest. Extracts were assayed with the Dual-Luciferase Reporter Assay Kit (Promega). **Results**. Quantitative PCR analysis showed that BeWoMDR cells express ~10-fold higher levels of P-gp mRNA than BeWo cells. Syncytialisation of BeWo and BeWoMDR cells with forskolin further increased P-gp mRNA by ~7-fold in each cell line. The elevated P-gp expression in BeWoMDR cells resulted in a reduced activation of the GR by ~50% ($P < 0.01$) in comparison to BeWo cells dosed with 10^{-9} M to 10^{-6} M dexamethasone. The activation of the GR by dexamethasone in both cell lines was GR-dependent, as response to dexamethasone was completely inhibited by co-incubation with RU486. **Conclusions**. These data support the hypothesis that P-glycoprotein is a component of the placental glucocorticoid barrier. Thus, 11β-HSD2 and P-glycoprotein are likely to act in unison to reduce fetal and placental exposure to maternal glucocorticoids and minimise their growth inhibitory actions. Potentially, abnormally low levels of placental P-gp expression could result in glucocorticoid-induced fetal growth retardation and the programmed adult-onset complications.

1. Atkinson, D. E., *et al.* (2003) *Am J Physiol Cell Physiol*, 285, C584-591.

P1-086

Epigenetic Regulation of Human Trophoblastic Cell Migration Fahimeh Rahnama, Farhad Shafiei, Peter D. Gluckman, Murray D. Mitchell and Peter E.Lobie. *Liggins Institute, University of Auckland. 2-6 Park Avenue. Grafton. Auckland. New Zealand*

Pivotal to successful mammalian reproduction is the ability of a developing embryo to implant to the uterine wall and establish a nutrient supply via placentation. Implantation requires a number of distinct cellular functions including attachment to the endometrial cell, spreading of the embryonic trophoblast and invasion of the trophoblastic cell into the endometrium. The trophoblastic ectoderm of the developing embryo is required for the initial attachment to, and later invasion into, the endometrial layer of the uterus; and the extent of the primary trophoblastic invasion determines later placental efficiency, fetal viability and performance. We have examined the potential epigenetic regulation of human trophoblastic migration and invasion by use of the trophoblastic derived BeWo cell line. Treatment of cells with an inhibitor of methylation, 5-aza-2'-deoxycytidine (AZA), resulted in a conversion of BeWo cell morphology to a non-migratory phenotype. This was exemplified by the ability of AZA to prevent BeWo cell migration in wound healing and transwell migration assays. AZA consequently inhibited BeWo cell invasion through reconstituted basement membrane. Examination of components of the adherens junction complex pivotal for determination of cell phenotype revealed that AZA specifically increased the mRNA level of E-cadherin and ?-catenin but not a-catenin and ß-catenin. AZA also increased the promoter activity of both E-cadherin and ?-catenin. Protein levels of both E-cadherin and ?-catenin were increased by AZA and AZA enhanced their localization to sites of intercellular contact. Forced expression of E-cadherin and ?-catenin abrogated BeWo cell migration indicative that repression of these genes was required for BeWo cell migration. Cellular methylation is mediated by specific DNA methylatransferases (DNMT1, DNMT3a and DNMT3b). siRNA mediated depletion of the individual DNMT molecules did not affect BeWo cell migration. However, inhibition of BeWo cell migration was observed with siRNA mediated depletion of both DNMT3a and DNMT3b. Trophoblastic migration is therefore dependent on methylation mediated by the combined action of DNMT3a and DNMT3b. Appropriate epigenetic regulation of early placentation will therefore be pivotal for fetal viability and performance.

P1-087

A New Mouse Model of Intrauterine Growth Restriction Caused by Yolk Sac Placental Dysfunction Junwu Mu, Dawei Qu and S. Lee Adamson. *Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Depts of Ob/Gyn and Physiology, University of Toronto, Toronto, Canada M5G 1X5.*

Background: The yolk sac placenta supports the growth and development of the embryo during the critical period of organogenesis in humans (<10 wk) and in mice (<15.5 days). In the mouse, the yolk sac circulation continues to term (18.5 days) whereas in the human, the yolk sac regresses by the end of the first trimester (12 wk). Thus it is clear that in the human, the yolk sac placenta is not required for normal growth and development during the fetal period (>10 wk). In this study, we ligated the vitelline circulation to the yolk sac during the latter half of gestation in mice to determine the role of the yolk sac placenta in supporting the growth and development of the mouse fetus. We hypothesized that yolk sac dysfunction would cause intrauterine growth restriction and/or compensatory placental overgrowth. **Methods**: Pregnant mice between day 12.5 and 16.5 of gestation were anesthetized with isoflurane, the uterus was exposed through a skin incision in the maternal abdomen, and the vitelline veins were visualized through the intact uterine wall. Embryos in each mouse were divided into three groups. In the ligation group, the vitelline vein was ligated with a silk suture by inserting a curved needle around the vessel through the intact uterine wall. In the sham group, the needle was inserted and ligature tied in a region devoid of vessels. The remaining embryos served as controls. A total of 21 pregnant mice with 252 embryos were studied. The mice were dissected at day 17.5 of gestation. Embryonic survival, and body and placental weights were recorded. **Results**: No embryos were alive at day 17.5 following vitelline vein ligation at day 12.5 and 13.5 (0/19). Nearly half died following ligation at day 14.5 (27/48) and the other half survived but were growth restricted by 24% relative to control. All embryos were alive at day 17.5 following ligation on day 15.5 or 16.5 but were growth restricted by 18% and 12% respectively. Amniotic fluid volume was visibly reduced in the ligation group. Placental weight was not affected. No differences in survival rates, or in body and placental weights were observed between the control and sham groups. **Conclusions**: We developed a new mouse model of intrauterine growth restriction caused by yolk sac placental dysfunction. The yolk sac circulation is essential for survival during embryonic organogenesis in the mouse, and later in gestation is required for normal fetal growth and amniotic fluid volume regulation. Ligation of the yolk sac placenta does not cause overgrowth of the chorioallantoic placenta although compensatory changes in chorioallantoic placental morphology may occur.

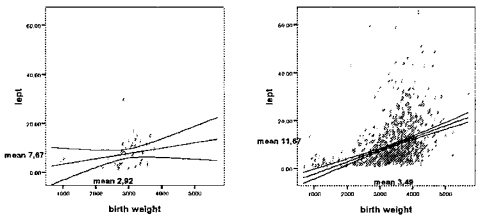
Supported by CIHR.

P1-088

Cord Leptin and Crossing Quartiles in Childhood *Bjoern Oeglaend, Lars Vatten. University Hospital Stavanger, University of Trondheim.*

Background: Epidemiological studies indicate that individuals born small and growing big in puberty have a higher risk of certain diseases. There is a possibility that children born small have biological mechanisms to preserve nutrition by means of organ and hormonal function and that this adaptive response can overshoot and cause obesity, insulin resistance and higher blood pressure. Leptin is one of the candidates for regulatory agent. Low leptin values are presumed to raise appetite and insulin activity. **Method**: In Stavanger we are in progress of a follow up study of 900 children; whereof 350 offspring of preeclamptic pregnancies and the rest offspring of normotensive pregnancies. Here we present preliminary data on leptin values in cord blood vs body size quartile crossing by age 10.8 years in some of the children. We define "quartile crossers" as children who cross 2 or more quartiles in weight for gestational age at birth and BMI at 10.8 years of age. **Results**: Leptin values at birth are significantly lower in quartile crossers (7,67; n=38 vs 11,49; n=175 p=0,013) and it is tempting to imply that leptin plays a role for regulating greater growth in these children. However, leptin value is associated with birth weight, and birth weight by itself could be a confounder since quartile crossers will have lower mean birth weight than non-crossers. (2,92 kg vs 3,49 kg p=0.000) When controlling for birth weight the association between quartile crossing and cord blood leptin is weaker. Bivariate correlation quotient between leptin values and "quartile crossing status" is -1,73 (p=0,013) , partial correlation quotient when controlling for birth weight -0,25 (p=0.73) For children born with birth weight under 50 percentile for gestational age, mean leptin value in crossers are lower than in not crossers (7,7 vs 8,6) but this is not significant with the numbers we so far have analyzed. The results illustrate the complexity and pitfalls in explaining associations between perinatal findings and findings later in life.

Figures:
scatter plot with regression line, 95% confidence interval and indication of mean birth weight and mean leptin values for the total cohort and for the quartile crossers respectively.



P1-089
Folate and Homocysteine Levels during Pregnancy affect DNA Methylation in Human Placenta and Infant Birth Weight Bohyun Park, Hyesook Park, YoungJu Kim, Hwayoung Lee, Eunhee Ha, Jungwon Min; Hun-jae Lee *Departments of Preventive Medicine, Ewha Womans University, Departments of Obstetrics and Gynecology, Ewha Womans University, Departments of Anatomy, Ewha Womans University, Medical Research Institute, Ewha Womans University, Department of Social Health, Inha University, In-cheon, Korea*

Background. DNA methylation is one of the best characterized epigenetic mechanisms that play a regulatory role in genome programming and imprinting during embryogenesis. In this present study, we investigate the association between DNA methylation in human placenta and maternal folate and homocysteine concentrations on the Methylenetetrahydrofolatereductase(MTHFR) genetic polymorphism during pregnancy and also evaluate the association between DNA methylation and adverse pregnancy outcome. **Methods.** We investigated 107 pregnant women who visited Ewha Womans University Hospital for prenatal care during 24-28 gestational weeks. During the second trimester, we measured serum homocysteine and folate concentrations. MTHFR 677 genetic polymorphism was determine by PCR-RFLP assay. The expression of DNA methylation in the human placentas was estimated using immunohistochemistry method. **Results.** Serum folate was negatively correlated with serum homocysteine concentration in all MTHFR genotype. We found positive correlation between folate concentrations and DNA methylation in human placenta($p<0.05$). Increasing concentration of homocysteine was associated with reduced DNA methylation in human placenta. The coefficient value was -2.03(-3.77,-0.29) in regression model($p<0.05$). Compared with infant's birth weight by DNA methylation expression status, the level of infant's birth weight were higher in women with above 90 percentile DNA methylation in placenta than in others ($p=0.001$) ***Conclusions.*** These findings suggest that maternal folate and homocysteine levels on the MTHFR 677 genetic polymorphism during pregnancy affect DNA methylation and adverse pregnancy outcome. "This work was supported by Korea Research Foundation Grant (KRF-2004-003-E00058)."

P1-090
Multidrug Resistance in Mouse Pregnancy S Petropoulos[1], GM Kalabis[1], W Gibb[4], SG Matthews[1-3] ; *Depts. of Physiology[1], Ob-Gyn[2] and Medicine[3], University of Toronto, Depts. of Ob-Gyn, Cellular and Molecular Medicine[4], University of Ottawa*

Background: Multidrug resistance genes (mdr1a and mdr1b) have been identified in rodents and together have been shown to functionally resemble human multidrug resistance gene Mdr1. It is thought that the mdr1a/1b gene product P-glycoprotein (P-gp) localized to the placental trophoblast serves to reduce transfer of xenobiotics and other substances including synthetic glucocorticoids, from mother to fetus; thereby reducing chemical exposure in the fetal compartment. We have previously shown that there are dramatic decreases in the expression of mdr1a/1b in the mouse placenta in late gestation suggesting an increased fetal susceptibility to the effects of xenobiotics in late gestation. The objective of this study was to elucidate the mechanism by which P-gp functions in the protection of the developing fetus. We hypothesized that placental P-gp is involved in excluding xenobiotics originating in maternal circulation from entering the fetal compartment and that this protection is greatest early in gestation. **Methods**: [3H]digoxin (0.05mg/kg) or [3H]dexamethasone (4μg/kg) were administered subcutaneously to pregnant FVB mice (n=6/group) on embryonic (E) days 12.5, 15.5 and 18.5. Both of these drugs are known to be substrates for P-gp. Mice were euthanized 1h post injection. Drug accumulation in the fetus was quantified following careful dissection using a Liquid Scintillation Counter. Radioactivity was normalized to maternal plasma levels to derive a drug ratio. **Results:** There was a significant decrease in accumulation of [^{3}H]digoxin in the fetal body at E18.5 when compared to E12.5 ($p<0.05$) and E15.5 ($p<0.01$). A significant decrease of [^{3}H]digoxin accumulation was observed in the fetal head at E18.5 when compared to E12.5 ($p<0.01$) and E15.5 ($p<0.01$). In contrast, accumulation of [^{3}H]digoxin in the amniotic fluid progressively increased throughout gestation. Similarly, [^{3}H]dexamethasone (4μg/kg) accumulation in both the fetal head and fetal body declined with gestational age, while accumulation in the amniotic fluid progressively increased. **Conclusion:** Although placental mdr1a/1b may protect the fetus from xenobiotics, the fetus itself may begin to express mdr1a/1b in late gestation as a compensatory mechanism for the decreased expression of placental mdr1a/1b thus enabling the exclusion of xenobiotics which have crossed into the fetal compartment from the maternal blood stream. This possibility is supported by the fact that substrate concentrations increase in the amniotic fluid. Understanding the mechanisms of transplacental drug transport is essential for the optimization of drug delivery regimens during pregnancy and for reducing the risk of toxicity to the developing fetus. **Supported by:** Canadian Institutes of Health Research (SGM)

P1-091
Cord Blood Amino Acid Concentrations are Paradoxically Increased, Not Decreased, in Growth Restricted Infants of Women with Preeclampsia Robert.W. Powers, Michael.P.Frank, Remya.A. Arul, and James.M. Roberts. Magee-Womens Research Institute and Dept. Ob/Gyn & Reprod Sciences, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213 USA

Background: Amino acids are a nutrient source for fetal growth, accounting for as much as 20 to 40% of fetal/placental energy requirements. There is active placental transport of amino acids from the maternal to the fetal circulation, resulting in a 2-fold increase in the concentration of most amino acids in the cord blood. Cord blood amino acid concentrations are decreased in intrauterine growth restricted (IUGR) infants, and this reduction in amino acids is thought to contribute to the restricted growth. Preeclampsia, like IUGR, is associated with reduced placental perfusion but is associated with reduced fetal growth in only about 1/3 of cases. **Objective:** The focus of this study was to compare maternal and fetal plasma amino acid concentrations in normal pregnancy, preeclampsia with growth restricted infants and pregnancies without preeclampsia but with growth restricted infants. **Study Design:** Maternal and cord blood EDTA plasma was collected at delivery from 39 nulliparous subjects, 25 women with normal pregnancy outcome, 7 with preeclampsia and growth restricted infants (gestational hypertension ≥ 140/90mmHg, proteinuria +2 random or 300mg/24hr, and gestational hyperuricemia), and 7 women without preeclampsia but with growth restricted infants. Amino acid analysis was performed by HPLC. Growth restricted infants were defined as infants with a birth weight less than the 5th centile corrected for sex, race and gestational age, and with a cord erythropoietin concentration >2sd above control. Statistical analysis was by ANOVA or sign test, and statistical significance accepted at $p<0.05$. **Results:** Maternal amino acids were significantly increased in women with preeclampsia (2382±556 μmol/L) compared to normal pregnant women (1952±287 μmol/L) and the women without preeclampsia but with growth restricted infants (1816±297 μmol/L, $p<0.05$). Surprisingly, despite a similar reduced birth weight, increased cord erythropoietin concentrations, decreased hPL and IGF-1, and increased IGFBP-1, cord blood amino acid concentrations were significantly increased in the growth restricted infants of preeclamptic mothers compared to controls (3614±785 vs. 3280±840 μmol/L respectively, $p<0.05$); and the growth restricted infants of women without preeclampsia exhibited the expected decrease in cord blood amino acids compared to controls (3093±226 vs. 3280±840 μmol/L respectively, $p<0.05$). **Conclusions:** These data suggest, that in contrast to IUGR, there may be enhanced availability and perhaps placental transport of amino acids and/or reduced fetal amino acid utilization during preeclampsia with growth restricted infants. These data also likely indicate basic differences in the pathophysiology of preeclampsia and IUGR both associated with reduced placental perfusion but with different maternal outcomes.

P1-092
Placental Growth and Fetal Growth: An Approach to Individualized Placental Growth-birth Weight Estimation Carolyn M. Salafia, Jun Zhang, Adrian K. Charles, Michaeline Bresnahan, Wenyu Sun, Patrick Shrout, Elizabeth M. Maas. Department of Epidemiology, Mailman School of Public Health, Columbia University College of Physicians and Surgeons, New York, New York; Epidemiology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD; Department of Pathology, Princess Margaret Hospital, Perth, Western Australia, New York State Psychiatric Institute, New York New York; Department of Psychology, New York University, New York, New York; EarlyPath Clinical and Research Diagnostics, Larchmont, NY

Background: Standard gross placental growth measures capture dimensions relevant to specific placental functions. Our objective was to determine their accountability for variance in birth weight, an important proxy for intrauterine "adequacy" in fetal origins studies. **Materials and Methods**: The sample consists of 24,152 singleton liveborn children of the Collaborative Perinatal Project delivered from 34-42 6/7 weeks gestation, with complete data for six placental growth measures (placental disk shape, umbilical cord length, distance from cord insertion to nearest margin, large diameter, small diameter, placental thickness) and placental weight. Contributions of placental growth measures associated with birth weight were examined with multiple linear regression. The model was applied to seven maternal characteristics (age, height, weight, parity, socioeconomic status, cigarette use, and race) to determine whether their known associations with birth weight are mediated by placental growth effects. **Results**: Placental weight alone accounted for 36.6 percent of birth weight variation; the six gross placental growth measures accounted for 28.1 percent. Combined, all placental measures accounted for 39.2 percent of birth weight variation. 13.9 percent of birth weight variance could be explained by the maternal characteristics (r^2= 0.139). After adjustment for the placental growth measures (disk shape, larger and smaller diameters, distance from the cord insertion to the placental edge, disk thickness, cord length and placental weight), the r^2 attributable to the maternal characteristics after adjustment for placental growth measures was 0.07. Thus 50.4 percent of the birth weight variance accounted for by these maternal characteristics appears to be mediated by their effects on placental growth variables.

Conclusions: Placental growth measures are highly predictive of birthweight. Evidence suggested that impact of all maternal characteristics except smoking was consistent with mediation by placental growth characteristics. Modeling of placental growth measures may help individualize long term health risks attributed to birth weight effects.

P1-093

Diabetes-induced Fetal Obesity is Associated with Placental Programming of Lipid Signals T. Radaelli *H. Ortega, * J. Minium, K. Farell, A. Varastehpour, E.Herrera, P. Catalano, S. Hauguel-de Mouzon. Department Reproductive Biology, MHMC at Case Reserve Western University, Cleveland, and *CEU, Madrid, Spain.

Diabetes is associated with exaggerated hyperlipidemia in the mother and higher rate of adiposity in the fetus. Increased transplacental transfer of lipid substrates at the time of exponential growth of the fetus may result in alterations of fetal adipogenesis. We investigated whether diabetes induces changes in placental lipids and how this can help understand the mechanisms resulting in excess fetal adiposity. **Methods:** Maternal, cord plasma and placenta were obtained from elective term Cesarean deliveries in women with normal and Gestational Diabetes Mellitus (GDM). Samples were classified into 2 groups according to neonatal body composition at birth. Body composition was assessed by anthropometrics. Placenta and plasma lipid profiles were measured by gas chromatography. Gene expression was monitored by oligonucleotide microarray analysis (Affymetrix U133A) of placenta from lean and obese neonates and real time RT-PCR. **Results:** Neonatal % body fat was 16.7±2.8 (n=8) in pregnancy with GDM and 7.4±1.9 (n=7) in controls. 45 genes (12 % of modified genes) related to energy metabolism were modified in placenta of obese neonates, 27 of which were specific to synthesis of triglycerides, cholesterol and eicosanoid precursors. The concentration of omega-3 polyunsaturated fatty acids (PUFA), docosahexaenoic (DHA) and eicosapentaenoic (EPA) acid was increased 2-fold in placenta of obese neonates with no modification of arachidonic acid or other omega-6 PUFAs. DHA concentration was increased in maternal (55±2.6 vs 43±3 mg/l, $p<0.05$) and umbilical plasma (36± 0.8 vs 25 ± 2.5 mg/l, $p<0.02$) of obese neonates compared with controls. The gene expression levels of the secretory phospholipases PLA2G2A and PLA2G5, rate limiting enzymes for PUFA hydrolysis were greater in placenta of obese neonates ($p<0.001$) By contrast the calcium independent isoform PLA2G6 was not modified. **Conclusion:** These data suggest that secretory phospholipases A2 are instrumental in increasing the concentration of omega-3 eicosanoid precursors in placenta of obese neonates. The enrichment in omega-3 vs omega-6 PUFAs may contribute to enhance fat accretion in the fetus through specific lipid signaling pathways.

P1-094

Excess Fetal Growth and Adiposity is Negatively Correlated with Placenta Corticotropin-releasing Hormone T. Radaelli, Sammy Usmani, J. Minium, A. Varastepour, *J. Lepercq, P. Catalano, S. Hauguel-de Mouzon ; Department of Reproductive Biology, Schwartz Center for Metabolism and Nutrition, MetroHealth Medical Center, Case Western Reserve University, Cleveland, Ohio, USA. * Obstetrics and Gynecology, Hopital Saint Vincent-de- Paul, Universite Paris 5, Paris, France

The corticotropin-releasing hormone CRH system is part of a vast network regulating energy metabolism. Several partners of the network which include CRH related peptides and receptors are regulated in response to food intake and level of adiposity. CRH, the principal modulator of the Hypothalamo-Pituitary Axis is expressed in the placenta which is the exclusive source of CRH in the fetal circulation. The aim of the study was to investigate placental CRH in relation to neonatal adiposity. **Methods** Placenta and maternal blood were obtained at delivery (38-40 weeks) in term control CTL (6), gestational diabetes GDM (7) and Type 1 diabetic T1D (9) pregnancies. Neonatal body composition was estimated by anthropometric measurements at birth. Expression of genes related to CRH system was performed by oligonucleotide microarray (Affymetrix U133A) of total placenta RNA and confirmed by real-time PCR. **Results** The maternal metabolic parameters were as followed. Plasma glucose (mg/dl) and insulin (uU/ml) were higher in GDM: 95±9, 59±18 and T1D 103±3, 20±5 compared to controls 70 ± 9, 12±5 uU/ml. BMI (kg/m2) were 23±2.3, 39±2.6, 21±2.2 in CTL, GDM and T1D respectively. Birth weights (g) were 3200±112 in CTL, 4042±471 in GDM, 4300±230 in T1D. Placental weight was significantly increased in GDM and T1D compared to CTL. CRH was detected in all placenta examined with a mean signal intensity of 18217±4341. By contrast, CRH binding protein and CRH receptor R1 and R2 were not detected in placental tissue. CRH expression was decreased 2.1 and 2 fold respectively in placenta of GDM and T1D. This was associated with higher neonatal adiposity based on increased ponderal index (3.2 ±0.1 and 3.4 ±0.1 g/cm3) in diabetics compared with 2.5 ±0.2 in controls. By contrast, CRH expression was unchanged in placenta of T1D with lean babies (PI =2.6±0.2). Changes in placental CRH were not correlated with maternal BMI suggesting that regulation of CRH expression is not directly related to maternal energy stores. **Conclusion** Placenta CRH expression is decreased in type 1 diabetic and GDM pregnancy with macrosomic neonates. We speculate that placenta CRH contributes to the regulation of energy deposition in utero as suggested in adult non-pregnant individuals. The physiological and molecular basis for the CRH changes and potential contribution to fetal fat accretion have to be further examined.

Prematurity

P1-095

Aortic Narrowing in School-Children Born Preterm Confined to Those Growth Restricted in Utero Anna-Karin Edstedt Bonamy[1], MD; Ana Bendito[1], MD; Ellika Andolf[2], MD, PhD; Mikael Norman[1], MD, PhD. Department of Woman and Child Health, Karolinska Institute[1], and Karolinska Institute at Danderyd Hospital[2], Stockholm, SWEDEN

Background: Young people born very preterm have reduced aortic size (1). Likewise, those growth restricted while in utero have narrower arteries at later follow-up (2). As preterm birth in many cases is preceded by fetal growth restriction, the aim of this study was to determine the selective contributions of these two perinatal risk factors for lasting aortic growth failure. **Methods and results:** Echo-tracking ultrasound was used to measure the end-diastolic diameter of the abdominal aorta in 58 school children aged 7-12 years: 19 born very preterm (=30 weeks of gestation) and small for gestational age (preterm SGA), 20 born very preterm and appropriate for gestational age (preterm AGA) and 19 term controls with normal birth weight. Brachial blood pressure (BP) was also recorded. Both before and after adjustment for age, height and gender, children born preterm SGA had a significantly narrower aorta than both preterm AGA and term controls (mean 8.1 vs. 8.8 and 9.0 mm respectively, $p = 0.05$). Systolic BP was significantly higher in children born preterm and in boys. Aortic diameter and BP did not correlate. **Conclusion:** Only children born small for gestational age have narrower abdominal aorta. This indicates that fetal growth-restriction in early pregnancy might be of greater importance than preterm birth for the risk of adverse aortic growth. **References** 1.Edstedt Bonamy AK, Bendito A, Martin H, Andolf E, Sedin G, Norman M. Preterm birth contributes to increased vascular resistance and higher blood pressure in adolescent girls. Pediatr Res, 2005; in press. 2. Brodszki J, Lanne T, Marsal K, Ley D. Impaired vascular growth in late adolescence after intrauterine growth restriction. Circulation. 2005 May 24;111(20):2623-8

P1-096

The Effect of Pre-term Birth on Nephrogenesis Lina Gubhaju, Anthony Zulli and Mary J. Black; *Department of Anatomy and Cell Biology, Monash University, Clayton, VIC, Australia; Department of Medicine, University of Melbourne, Austin Health, Heidelberg, VIC, Australia*

Background & Aim Nephrogenesis, the formation of nephrons in the kidney, occurs mainly during late gestation of pregnancy in humans with no new nephrons formed after term. Hence, it is imperative to investigate the effects of pre-term birth on kidney development. We have recently shown that the baboon is a suitable experimental model of human nephrogenesis. The aims of the present study were to morphologically assess whether nephrogenesis continues after premature delivery in pre-term baboons and if so, to determine whether nephrogenesis/glomerulogenesis is affected by premature delivery. **Methods** Delivery and care of all fetal baboons were undertaken at the Southwest Foundation (San Antonio, Texas, USA). Fixed kidneys from premature fetal baboons (n = 8) delivered at 125 days gestation and ventilated for 6, 14 and 21 days were morphologically assessed and compared to kidneys of fetal baboons from normal gestation (n = 8) delivered at 125, 140, 175 and 185 days gestation. Immunohistochemistry was also undertaken using the endothelial cell marker, CD31, to examine glomerular vascularization. Nephron number was estimated using unbiased stereological techniques. **Results** Morphological assessment confirmed that nephrogenesis in the baboon was on-going at 125 and 140 days during normal gestation and was complete by 175 days gestation. Importantly, morphological assessment confirmed that nephrogenesis continues after premature delivery until 21 days. Nephron endowment in pre-term fetal baboons did not appear to be compromised. There was also a highly significant correlation between kidney weight and nephron number in the kidneys from fetal baboons in normal gestation (R^2=0.918, P=0.0002) and in the kidneys from prematurely delivered baboons (R^2=0.750; P=0.001). However, an interesting observation from the current study was the appearance of apparently abnormal glomeruli in kidney sections from the premature baboons. These glomeruli in the outer renal cortex exhibited a dilated Bowman's space and a shrunken glomerular tuft. Immunohistochemical studies with CD31 demonstrated that these glomeruli were not well vascularized. **Conclusions** In conclusion, these findings demonstrate that although nephron endowment may not be compromised in premature neonates it appears that there may be some pathological changes in the kidneys possibly due to abnormal vascularization of the glomeruli.

P1-097

Aberrant Glucose Tolerance in Adults with Birth Weight Below 1500g Petteri Hovi, MD [1,2], Sture Andersson, MD, PhD [1], Johan G Eriksson, MD, PhD [2], Anna-Liisa Järvenpää, MD, PhD [3], Sonja Strang, MD[1], and Eero Kajantie, MD, PhD [1,2]. *[1] Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland, FIN-00029, [2] Department of Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland, FIN-00300, and [3] Helsinki City Maternity Hospital, Finland, FIN-00029.*

Background: The association between small size at birth and impaired glucose regulation later during life is well established in subjects born at term. Association between very low birth weight (VLBW, birth weight < 1500g) and insulin resistance has, until now, been reported in prepubertal children. Whether this association persists into adulthood has not been studied. **Objective:** To assess glucose tolerance and

insulin sensitivity in young adults born VLBW. **Design/Methods:** We performed a 75 g oral glucose tolerance test in **243** adults aged 19 to 25, of whom **137** were born VLBW. Percentage of males was 43% in the VLBW group and 39% in the control group. Among the VLBW infants gestational age ranged between 24 and 35 weeks (mean 29 and SD 2.4) and birth weight SD score median was -1.0, interquartiles were -2.6 and 0.0. Term born control subjects were matched for age, gender, and birth hospital. **Results:** Adjusted for confounding factors 2-hour glucose was 0.3 mmol/l higher in the VLBW group (P= 0.03, see table). Excluding persons born with birth weight <= -2.0 SD (SGA) did not affect the result (difference 0.4 mmol/l, p=0.03). Impaired glucose tolerance (2-hour glucose >= 7.8 mmol/l) was detected in 9 VLBW and 4 control subjects (p=0.4).As compared to the term born control group, VLBW adults fasting insulin was 14% higher (p=0.03) and 2-hour insulin was 47% higher (p<0.01, see table). After exclusion of persons born SGA, VLBW adults still had higher 2-hour insulin by 40% (p<0.01). **Conclusions:** Young adults born as VLBW infants had higher insulin levels and 2-hour glucose levels as compared with term-born subjects. This suggests that as well as being born small at term, also being born severely preterm may have long-term effects on glucose regulation.

	VLBW	Control	Difference*	95% CI for difference*		p*
Fasting glucose mmol/l	4.7	4.7	0.0	-0.06	0.12	0.52
2-hour glucose mmol/l	5.5	5.2	0.3	0.04	0.62	0.03
Fasting insulin mU/l	5.6**	5.1**	14%	2%	26%	0.03
2-hour insulin mU/l	35.2**	23.6**	47%	27%	67%	< 0.01

* values are adjusted for age, sex, parental diabetes and adult BMI.
** values are geometrical means.

P1-098

Plasma Adiponectin and Leptin Levels of Low Birth Weight Infants in the First Two Years of Life Makoto Inoue, Kazuo Itahashi, Toshio Takeuchi, Katsumi Mizuno, Jyunya Iwasaki *Department of Pediatrics, School of Medicine, Showa University, Tokyo, Japan.*

Objectives: Several investigators reported that higher concentrations of plasma adiponectin in newborn infants than those in adults and its levels were related to fetal growth. The aim of this study was to examine plasma adiponectin and leptin concentrations of low birth weight infants and their correlations with anthropometric parameters in the first two years of life. **Methods and Procedures**: Blood samples were obtained once from 39 low birth weight infants from 2 to 24 months of postmenstrual age (17 males and 22 females, gestational age 24.1-38.1 weeks, birth weight 517-2,402g, 20 SGA infants). The adiponectin and leptin concentrations were determined by enzyme-linked immunosorbent (ELISA) assays using commercially available kits. **Results**: The plasma adiponectin and leptin concentrations were 23.8 +/- 10.3 mcg/ml, and 3.1 +/- 4.0 ng/ml, respectively. The concentrations of adiponectin and leptin did not differ significantly between SGA and AGA infants. There was no significant gender difference in adiponectin and leptin concentrations. Plasma adiponectin levels had significantly negative relationships with body weight (r=-0.33, p<0.05) and body length (r=-0.45, p<0.01), controlling for postmenstrual age, gestational age and birth weight. Plasma leptin concentrations correlated positively with body mass index (r=0.46, p<0.001), controlling for these factors. There was no relationship between adiponectin and leptin concentrations. **Conclusions**: These results indicate that the leptin levels may reflect adiposity and the adiponectin levels may be inversely correlated with postnatal growth of low birth weight infants during 2 years of postmenstrual age.

P1-099

Prematurity and Intrauterine Growth Retardation Predispose to Hypertension, Renal Function Loss and Microalbuminuria at Age 20 Mandy G. Keijzer-Veen, Jeroen Nauta, Friedo W. Dekker, Bert J. van der Heijden, *Department of Pediatric Nephrology, Erasmus MC – Sophia, Rotterdam and Department of Clinical Epidemiology, Leiden University Medical Center, The Netherlands.*

Background: Preterms and intra-uterine growth retardated (IUGR) subjects are presumed to be at risk to develop hypertension and renal disease at adult age. Small differences may already be present at young age. Aim: This study was conducted to evaluate 24-hour ambulant blood pressure values, renal function and renal functional reserve capacity at 20 years of age in very preterm born subjects, both appropriate and small for gestational age, and controls. **Methods**: We included 52 premature subjects (gestational age < 32 weeks: POPS-database, the Netherlands) divided in 29 appropriate and 23 small for gestational age (AGA, SGA) and 30 at term born controls (Co, 37-42 weeks) at age 20. Blood pressure was obtained with a 24-hour blood pressure monitor. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) was measured by Inulin and ?-amminohippuric acid (PAH) clearances (ml/min/1.73m^2) before and after kidney stimulation (low dose dopamine infusion (2 µg/kg/min) and oral protein intake). Albumin creatinine ratio (ACR: mg/mmol) in three spot urines was measured. Differences in systolic and diastolic blood pressure (SBP, DBP), ACR, GFR and ERPF between the three groups were calculated with a linear regression analysis.**Results** (table 1): SBP during the day was 4.2 mmHg higher in AGA subjects than in Co (95%CI 0.2;8.0). Daytime DBP and nighttime BP values did not differ between groups. According the JNC 7 criteria, prehypertensive stage or hypertension was 1.6 times (95%CI 1.0;2.4) more prevalent in preterms (both SGA and AGA) than in Co. At baseline GFR did not differ between the three groups, but ERPF was 71.3 ml/min/1.73m^2 lower in SGA compared to Co. After renal stimulation GFR and ERPF increased significantly with 15.3 and 269.3 ml/min/1.73m^2 resp. (both P < 0.001). Stimulated GFR tended to be lower in SGA compared to Co (118.7 resp. 131.5 ml/min/1.73m^2). SGA subjects tended to be less capable of increasing GFR after renal stimulation than Co. ERPF remained lower in SGA than Co after renal stimulation (747.9 resp. 878.1 ml/min/1.73m^2). Microalbuminuria (ACR > 2.2) was only present in 2 SGA subjects. High normal microalbuminuria (ACR 1.1-2.2) was present in 2 SGA and 1 AGA subjects, but none of the Co. The mean logarithmic ACR was significantly higher in SGA subjects compared to Co (0.37 resp. 0.22 mg/mmol). **Conclusion**: Increase in SBP and decrease in renal function are present at young adult age in subjects born very premature. These data support the hypothesis that IUGR subjects in preterm subjects are at risk to develop renal disease and hypertension in adult life. Follow-up of these preterm subjects is recommended to evaluate further decrease in renal function.

	SGA (n=23)	AGA (n=29)	Co (n=30)	SGA vs. Co P value
SBP day*	122.7	123.1 †	119.6	0.111
DBP day*	73.0	72.2	71.7	0.604
ACR (geometric)	0.37	0.28	0.22	0.028
GFR baseline	107.1	116.1	111.6	0.408
GFR stimulated	118.7	130.1	131.5	0.077
Increase GFR	11.6	13.9	19.8	0.098
ERPF baseline	514.8	533.9	586.1	0.034
ERPF stimulated	747.9	808.1	878.1	0.024
Increase ERPF	233.1	274.2	291.9	0.188

Table 1: Means for blood pressure (mmHg), ACR (mg/mmol), GFR and ERPF (ml/min/1.73 m^2) in the study groups and p-value for difference between SGA and Co. * Adjusted for gender. † p-value for AGA vs. Co = 0.042.

P1-100

Decreased Kidney Size at 20 Years of Age in Subjects Born Very Prematurely Compared to at Term Born Controls Hilda A Kleinveld, Mandy G Keijzer-Veen, Annick S Devos, Friedo W Dekker, Bert J van der Heijden, *Department of Pediatric Nephrology and Radiology, Erasmus MC – Sophia, Rotterdam and Department of Clinical Epidemiology, Leiden University Medical Center, The Netherlands.*

Background: Intrauterine growth retardation is presumed to be associated with decreased renal size and impaired renal function as a result of impaired kidney development and nephron deficit. It is not known whether very preterm birth also affects renal size at adulthood. We investigated the effect of both intrauterine growth retardation and gestational age on kidney size 20 years after birth. **Methods**: In very premature subjects (< 32 weeks of gestation), either small or appropriate for gestational age (22 SGA and 29 AGA), and 30 at term controls ultrasonic measurements of bipolar kidney length and kidney volume were taken from both left and right kidneys 20 years after birth. Relative kidney length (RKL) and volume (RKV) were calculated by respectively dividing kidney length by body height, and kidney volume by body surface area. Subjects were selected from the Project Of Prematures and Small for Gestational age infants (POPS) cohort. Data were analysed using a linear regression model. **Results**: Both absolute and relative left kidney length and volume were significantly lower in preterm subjects compared to controls. Left kidney length was 1.0 cm smaller in SGA compared to controls (95% CI 0.5;1.5) and 0.7 cm smaller in AGA compared to controls (95% CI 0.2;1.2). Left RKL was 0.3 cm/m smaller in both SGA and AGA compared to controls (95%CI 0.0;0.6).
These differences were more obvious is females than in males. No significant differences were found in both absolute and relative right kidney size between the three groups. Between SGA and AGA subjects no differences in kidney size was found. In 70.0% of controls left kidney was larger than right, compared to 40.9% in SGA (RR 1.7 95%CI 1.0;3.0) and 48.3% in AGA (RR 1.5 95%CI 0.9;2.3). **Conclusion**: In our study very preterm subjects have smaller kidneys at the age of 20 years. No relation between intra uterine growth retardation and renal size was found within our preterm subjects. Our data suggest that kidney development is impaired after preterm birth.

P1-101

Blood Pressure in Young Adults with Birth Weight Below 1500 g Sonja Strang, Petteri Hovi, Sture Andersson, Anna-Liisa Järvenpää, Johan Eriksson, Eero Kajantie; *National Public Health Institute, 00300 Helsinki, Finland; Hospital for Children and Adolescents, Helsinki University Central Hospital, 00029 HUS, Helsinki, Finland*

Background. It is well established that slow intrauterine growth in term-born males and females is associated with elevated blood pressure in adulthood. Severely preterm birth is followed by a period of prematurity-associated illness and disrupted growth. However, only a small number of studies have assessed blood pressure in adults born severely preterm. **Methods.** The preterm subjects belong to a cohort of very low birth weight (VLBW; <1500 g) infants born 1978-1985 and treated at Helsinki University Central Hospital. Their mean birth weight was 1126 g (SD 216), gestational age 29.3 wks (SD 2.3) and relative birth weight (adjusted for gestational age and sex according to Finnish standards) -1.3 SD (SD 1.6). The preterm group was compared with healthy controls born at term (=37 weeks of gestation), matched for sex, age and birth hospital, whose mean birth weight was 3580 g (SD 485), gestational age 40.1 wks (SD 1.2) and relative birth weight 0.0 SD (SD 1.1). In the morning after an overnight fast, the

subjects attended a clinical examination which included measurements of height, weight and blood pressure (the mean of two recordings by an automatic sphygmomanometer after a 15 min rest). All correlations are adjusted for current age and BMI. The study is ongoing and the results are based on the first 130 subjects and 93 controls. **Results.** At 18 to 25 years of age, VLBW-born subjects were shorter than those born at term (males: 174 vs. 182 cm; p<0.0001; females: 163 vs. 167 cm; p=0.005). BMI was lower in VLBW-born males (21.8 vs. 23.7 kg/m^2; p=0.02) but not in females (p=0.6). Systolic and diastolic blood pressure was associated with BMI in both sexes (r=0.18 to 0.30; p=0.04) but was unrelated to height. VLBW birth was associated with higher systolic and diastolic blood pressure among females but not among males (Table). Further analysis among VLBW-born subjects showed that, among females but not among males, higher blood pressure was associated with lower gestational age at birth (one wk of decrease in gestational age corresponding to 1.5 mmHg, 95% CI 0.3 to 1.6, increase in systolic and 1.1 mmHg, 95% CI 0.2 to 2.0, increase in diastolic blood pressure). Relative birth weight (adjusted for gestational age was not related to blood pressure. Excluding subjects with relative birth weight <-2 SD did not alter the results. **Conclusions.** Severely preterm birth is associated with a higher clinic-measured blood pressure in young female adults. This association is not seen among males. The results reinforces previous suggestions that being born preterm may have similar long-term consequences on blood pressure as being born small at term. Reasons for the sex difference remain to be elucidated.

Table. Blood pressure (mmHg) at age 18 to 25 years in subjects born with very low birth weight
(VLBW; <1500 g) and term-born controls

	Males				Females				
	VLBW (n=55)	Control (n=38)	Mean difference* (95% CI)	p*	VLBW (n=75)	Control (n=55)	Mean difference* (95% CI)	p*	p for interaction*†
Systolic	125.9	128.4	-0.7 (-5.8 to 4.4)	0.8	117.3	112.3	5.2 (1.4 to 9.0)	0.008	0.03
Diastolic	79.2	78.8	1.6 (-1.8 to 4.9)	0.4	78.7	74.7	4.3 (1.1 to 7.4)	0.009	0.2

* Adjusted for current age and BMI. † Interaction sex*VLBW birth.

Reproductive System Outcomes

P1-102

Relationship Between Maternal Psychosocial Status and Pregnancy Outcomes
Dabbaghi, F. Ph.D[1] Sadeghi, H. MS [2] Jahaanfar, SH. Ph.D[1] Haghani, H. MS[3]

Pregnant mothers with high anxiety level are likely to produce who are hyperactive and irritable, have sleep disorders and low birth weight newborn. The main objectives of this study were to examine the correlation between psychosocial status of mothers during pregnancy and pregnancy outcomes. The present research is comparative study. In order to collect information questionnaire consisted of individual characteristics and a data record form included pregnancy outcomes (L.B.W, IUGR, preterm labor) were used. The questionnaires were completed at 25-29 weeks of gestation by the participants, and the data record forms were filled out by the researcher after the delivery of participants were completed. Three hundred and sixty subjects including nuliparas and multiparas (Para 0-4) from two population groups (women with appropriate and inappropriate psychosocial status). Who were classified using a psychosocial status questionnaire (Copper 1996) were selected through consecutive sampling method (N=180 for each group). Three health centers and the delivery ward of Mobini hospital of Sabzevar, were chosen for the study. Findings indicated that psychosocial status of mothers were associated with L.B.W (P=0/005), IUGR (P=0/028) and preterm labor (P=0/00). Also finding showed that mothers occupational status and montly family income were 2 variables with confounding effect on pregnancy outcome within both groups. Our suggests that women with poor psychosocial status are at increased risk for L.B.W ,IUGR and preterm labor, therefore interventions such as emotional support by family and health care providers should be targeted at these women to relive their emotional distress and also to enhance their self-steem and confidence. **Key words**: Pregnant mothers, psychosocial status, Pregnancy outcome.

1- Assistant professor, school of nursing and midwifery, Iran university of medical sciences and health service, Tehran, Iran (Corresponding author) 2- Ms in midwifery 3.- Assistant professor, school of nursing and midwifery, Iran university of medical sciences and health service, Tehran, Iran; 4- Senior lecture. Faculty of management & medical information sciences, Iran university of medical sciences and health services, Tehran, Iran.

P1-103

Association Between Agricultural Pesticide Applications and Spontaneous Abortion Among Farmers Mina Iravani (MS) *School of Nursing and Midwifery , Islamic Azad University . Dezful . Iran*

Introduction : Abortion is an integral part of the reproductive health care continuum . Occupational exposures to pesticides may increase parental risk of early pregnancyloss; although some show an association, findings have not been consistent. **Objective :** The aim of this study was association between agricultural pesticide applications and spontaneous abortion in parents who are farmers. **Materials and Methods :** This study was an case – cohort study that undertaken of March 2003 to March 2005 in Iran.cases were 350 pregnant women who attended to health centers in village. For each case one control was selected . Age of women , gravida , female education , history of abortion , economical condition were matched between cases and controles. The questionnaire was used to abtain information about parents and risk factors. In this study the frequency of spontaneous abortion in parents who were exposed to agricultural pesticide during the period of preimplantation- organogenesis compared with non-exposed parents. Statistical methods for normally distributed variables included 2-tailed student t-test and chi-squar analysis. The accepted level of significant was p< 0.05. **Results :** The results this study showed that the frequency of abortion in women who were exposed to agricultural pesticide during early pregnancy was increased compared with control group (30.6% vs 18.5% , p < 0.05). The frequency of abortion in women who their husbands were exposed to agricultural pesticide during the period of preimplantation was increased compared with control group (28.2% vs 20.4% , p < 0.05). The frequency of abortion in women who themselves and their husbands were exposed to agricultural pesticide (during the period of preimplantation- organogenesis) was increased compared with control group (35.2% vs 21.6% , p < 0.05). **Conclusion :** This study suggest that parents working in areas of high pesticide application are at increased risk for spontaneous abortion .

P1-104

Intergenerational Effects of Parental Birthweight on Offspring Birthweight from a Population-based Study in South India G Priya[1], B Antonisamy[1], P Raghupathy[2]
Departments of Biostatistics[1] and Child Health[2], Christian Medical College, Vellore, India

Background: The birthweights of babies has been documented in several intergenerational studies to be strongly related to those of their maternal birthweight. There is a dearth of information on this aspect of fetal growth in developing countries, besides published studies from Guatemala and Mysore, India. **Objectives:** This paper aims to examine the evidence and magnitude of the intergenerational relationship between the birth size of parents and that of their offspring in a developing country. **Methods:** This study was carried out in Vellore and an adjoining rural area. The parents were drawn from an original birth cohort of 1969-1973. We traced 422 mothers with 391 sons and 381 daughters (a total of 772 mother-offspring pairs) and 473 fathers with 381 sons and 379 daughters (a total of 760 father-offspring pairs). The spouses of these mothers and fathers did not belong to the cohort, their birth and longitudinal data were unavailable and hence not studied. Of 895 parent-child pairs studied, 51% of the parents had more than one child. Details of birth characteristics, adult size, education and material possessions were available for the parents. Offspring details of gestational age, date of birth, birth order, maternal antenatal events, anthropometry at birth and current anthropometry were collected. Relationship between parent and offspring measurements were examined using random effects regression models, allowing parents having more than one child in the study. **Results:** The prevalence of low birth weight (LBW) in daughters and sons was 19.7% and 15.2% as compared to mothers (21.1%) and fathers (18.6%) respectively. The mean (SD) birthweight was similar between fathers-sons pairs (2847g (489); 2853g (485), P=0.8), and mothers- daughters pairs (2791g (445); 2749g (482), P=0.1). Maternal birthweight (152g/1 kg; P=0.002) and paternal birthweight (179g/1kg; P<0.001) were significant predictors of their offspring birthweight after adjusting for gestational age, sex of the offspring, order of the offspring, BMI, height and education. Each cm increase in fathers' and mothers' height resulted in an increase of 7.6g (P=0.03) and 11.5g (P=0.004) in offspring birthweight respectively after adjusting for the confounding factors. Mothers who had LBW were 1.8 (95% CI: 1.01 – 3.05) times more likely to deliver a baby with LBW as compared to mothers who weighed >2500g after adjusting for sex of the offspring, gestational age and parity. Similarly LBW fathers had 1.8 (95% CI: 0.77 – 4.09) times higher risk of producing babies with LBW as compared to fathers weighing >2500 g at birth. Heavier and taller parents are less likely to produce LBW babies. **Conclusion:** Parental birthweight status appears to affect the birthweights of their offspring with higher birthweights of parents being significantly related to the birthweights of offspring. The adult weight and stature of the parents also positively influenced the birthweights of their offspring. Mother, father and offspring will need to be studied in the same model to determine the magnitude of each parent towards the offspring birthweight.

P1-105

The Polycystic Ovary Syndrome (PCOS) in Adolescence and Intrauterine Exposure to Maternal Androgens Roger Hart[*], Deborah M Sloboda[**], Robert Norman[***], Dorota Doherty[*], Martha Hickey[*]; [*] *School of Women's and Infants' Health, The University of Western Australia, Perth, Australia; [**]Women and Infants Research Foundation, King Edward Memorial Hospital, Subiaco, Australia; [***] Research Centre for Reproductive Health, University of Adelaide, South Australia.*

Background: PCOS is the most common female endocrinopathy and affects up to 10% of reproductive age women. PCOS has profound endocrine, reproductive, metabolic and cosmetic consequences, and carries an increased risk of diabetes and possibly of cardiovascular disease. The origins of PCOS are unclear and despite epidemiological evidence suggesting a genetic contribution, the genetic determinants remain unknown. Current hypotheses suggest that PCOS originates from intrauterine and early childhood events, which alter function of the hypothalamic pituitary ovarian axis. Female exposure to male levels of testosterone in utero, in both non-human primates and sheep, show clinical and biochemical features of PCOS including; hypersecretion of lutenizing hormone; abnormal insulin secretion/action and hyperandrogenic anovulation. In both species, prenatal androgenization produces

enlarged ovaries with multiple antral follicles. We hypothesise that increased fetal exposure to androgens will programme the subsequent development of PCOS in adolescence. **Methods: Diagnosis of PCOS:** A unique cohort of 850 West Australian girls followed prospectively from 10 weeks gestation until adolescence (The Raine Cohort), were assessed in the early follicular phase of their menstrual cycle, within the first 2 years since menarche. The diagnosis of PCOS was defined as *per* the Rotterdam Consensus as *the presence of ≥2 of the following criteria: polycystic ovaries, oligo/anovulation or clinical or biochemical evidence of hyperandrogenism.* **Results:** Preliminary data from 42 girls indicates a trend linking PCOS in adolescence with increased maternal serum testosterone levels at 18 weeks of pregnancy (2.7±0.83nmol/l *vs* 3.3±0.80nmol/l, Figure 1). No association between maternal DHEAS or androstenedione and subsequent PCOS in her offspring were evident.

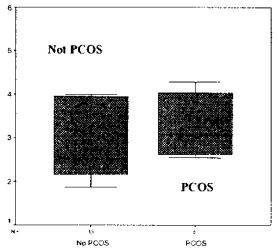


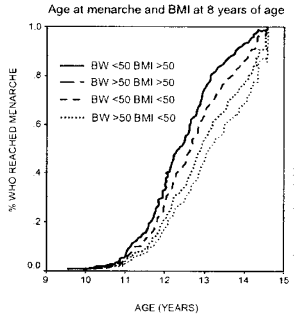
Fig 1. Maternal serum testosterone, (nmol/L), at 18 weeks of gestation, in relation to the presence of PCOS in their daughters.

Conclusions: These preliminary results support animal data indicating that increased fetal exposure to testosterone may lead to a subsequent PCOS phenotype in the offspring. The hypothesis has not previously been explored due to lack of suitable human cohorts and has substantial implications for the understanding of PCOS and its potential prevention. The study is ongoing and more data will be available for presentation.

P1-106

Age at Menarche is Related to Birthweight and Postnatal BMI in a Cohort of Australian Adolescents Deborah M Sloboda[1,2], Roger Hart[1], Dorota Doherty[1,2], Craig Pennell[1], Martha Hickey[1]; [1]*School of Women's and Infants' Health, The University of Western Australia, Perth, WA;* [2]*Women and Infants Research Foundation, Subiaco, WA*

Background: Subtle changes in the intrauterine environment may determine fetal development as well as adult health. It is highly likely that reproductive function (menarche, menstrual disorder, menopause and PCOS) may also be programmed *in utero*. Low birth weight and accelerated catch-up growth may be a markers of an adverse intrauterine environment and are associated with changes in the timing of adrenarche and menarche alterations in ovarian responsiveness, follicular development. In this study we determined the association between birth weight and postnatal growth and the age at menarche. ***Methods:*** A unique cohort of 860 West Australian girls was followed prospectively from 10 weeks gestation until adolescence (13 years of age). Birth weight and length, and weight and height during childhood were recorded. 349 girls in the cohort completed a puberty questionnaire which included age at menarche. Kaplan-Meier survival probabilities and log-rank tests were used to estimate median age at menarche. Multivariable Cox regression analysis was performed to evaluate associations between fetal and postnatal growth and age of menarche. Fetal growth was summarised using expected birthweight ratio (a ratio of infant's birthweight over median birthweight) adjusted/appropriate for maternal height, sex, nulliparity and gestational age. Postnatal growth is expressed as body mass index (BMI). ***Results:*** The median age of menarche within this subset of 349 girls was 12.6 years (range 9.4-14.6). Expected birthweight ratio ($p<0.008$) and BMI at 8 years of age ($p<0.001$) were both associated with age at menarche. Expected birthweight ratio less than median (BW<50) was associated with early menarche (hazard ratio, (HR) 1.28, 95% CI 1.04-1.59). BMI greater than the median (BMI>50) at 8 years of age was also independently associated with early menarche (HR 1.73, 95% 1.36-2.22). The combination of BW<50 and BMI>50 was associated with the earliest age at menarche (53% by 12.6 years). This was followed by in order: girls born BW>50 and BMI>50 (44% by 12.6years); girls born BW<50 and BMI<50 (34% by 12.6years); finally the girls born BW>50 and BMI<50 (28% by 12.6years) (see figure).



Conclusions: We have shown in this contemporary cohort of relatively well-nourished children in Western Australia that early age of menarche is independently associated with both birthweight and BMI at 8 years of age. This association is strongest in those girls who were of relatively low birth weight and demonstrated higher postnatal weight gain. These data therefore may imply that early life events play an important role in subsequent reproductive development.

P1-107

Effects of Maternal Protein Restriction During Pregnancy and Lactation on Cyclicity and Fertility of the Female Offspring Elena Zambrano[1], Carolina Guzmán[1], Guadalupe Rodríguez[1], Fernando Larrea[1], Peter Nathanielsz[2]. [1]Department of Reproductive Biology, Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubirán, Mexico City, Mexico; [2]Center for Pregnancy and Newborn Research, University of Texas Health Sciences Center San Antonio, TX, USA:

Background. Studies in rats have shown that undernutrition during early stages of life delays sexual development (*Biol Reprod. 2003. 68:390*), while in the sheep model it impairs fetal ovarian development and reduces female fertility rate in adulthood *(Anim Sci. 1995. 60:223).* **Methods.** Female Wistar rats were mated and fed either a Control 20% Casein (C) or Restricted 10% Casein (R) diet during pregnancy and/or lactation providing four groups, CC, RR, CR and RC (pregnancy first letter, followed by lactation – second letter). After weaning all female pups were fed the control diet. At 20 weeks, 1 year and 22 months of age, vaginal smears were collected daily over 10 days and vaginal cytology was analyzed to determine the cyclic patterns in each group. Fertility was evaluated at the ages of 20 weeks and 1 year by placing females in pairs with one proven male breeder during 5 or 7 days respectively. Cycle lengths are presented as mean ± SEM. Statistical analysis was performed using ANOVA. Fertility rate was expressed as percentage, differences were calculated by $?^2$. n = 4 to 5 litters, $p<0.05$. **Results.** Fertility was closely related to cyclicity. At 20 weeks, a time at which fertility was maximal in all groups (table 1), cycles were clearly discernible in all animals in all groups, RC pups presented longer cycles in comparison with the rest of the groups (Cycle length in days CC=4.6±0.2a, RR=4.9±0.1a, CR=4.9±0.3a and RC=6.3±0.5b). By 1 year, cycle length was unchanged in CC (6.7±0.9d) and RC (7.4±1.7d) but had increased in RR (9.7±0.9d) and CR (7.5±0.7d). However, the cycle length did not differ between the three experimental groups and controls. No cycle lengths were calculated at 22 months post natal age because only one animal in the CC and one animal in the CR groups demonstrated cycles (Fig 1). Fertility rate at 1 year had fallen in all restricted groups (Table 1). When compared within group at different ages, premature cyclicity and fertility loss were observed in all restricted groups ($p<0.05$). **Conclusion.** Maternal protein restriction during early development programs ovarian function, producing premature loss of cyclicity and fertility in the rat.

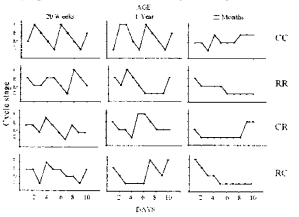


Fig. 1. Representative Estrous cycle of each group at different ages. Groups are as described in text; P: proestrous, E: estrous, M: metaestrous, D: diestrous.

GROUP	CC	RR	CR	RC
% Fertility 20 weeks	77.7	100	86.4	100
% Fertility 1 year	75 a	60 a.b*	50 b*	25 b*

Table 1. Fertility rate at 20 weeks and 1 year of age. Groups are as described in text. $p<0.05$ for data among groups not sharing at least one letter; $p<0.05$ * vs same group at 20 weeks of age.

P1-108

Childhood IQ in Relation to Obesity and Weight Gain in Adult Life Tarani Chandola, Ian J. Deary, David Blane, G. David Batty. *Department of Epidemiology and Public Health, University College London, London, UK; Department of Psychology, University of Edinburgh, Edinburgh, UK; Department of Primary Care and Social Medicine, Imperial College London, London, UK; MRC Social & Public Health Sciences Unit, University of Glasgow, Glasgow, UK*

Background. Recent studies suggest an inverse association between childhood IQ test scores and risk of total mortality in later life. This association could be mediated via established adult risk factors for mortality, for example obesity. In the present analyses, we examine the relation of childhood IQ test results with obesity in middle-age and weight gain across the life course. **Methods.** We analysed data from the National Child Development Study, a prospective cohort study based on 17,414 births to parents residing in Great Britain in the late 1950s. Childhood IQ was measured at age 11 and body mass index (BMI), an indicator of adiposity, was assessed at 16, 23, 33 and 42 years of age. Logistic regression (in which BMI was categorised into obese and non-obese) and structural equation growth curve models (in which BMI was retained as a continuous variable) were used to estimate the relation between childhood IQ and adult obesity, and childhood IQ and weight gain, respectively. **Results.** In unadjusted analyses, lower childhood IQ scores were associated with an increased prevalence of adult obesity at age 42. This relation was somewhat stronger in women ($OR_{per\ SD\ decrease\ in\ IQ\ score}$ [95% CI]: 1.38 [1.26, 1.50]) than men (1.26 [1.15, 1.38]). This association remains statistically significant after adjusting for childhood characteristics, including socio-economic factors, but was heavily attenuated following control for adult characteristics, particularly education (women: 1.11 [0.99, 1.25]; men: 1.10

[0.98, 1.23]). When weight gain between age 16 and 42 was the outcome of interest, structural equation modelling revealed that education and dietary characteristics in adult life mediated the association with childhood IQ. **Conclusions.** A lower IQ score in childhood is associated with obesity and weight gain in adulthood. In the present study, this relation appears to be substantially mediated via educational attainment and the adoption of healthy diets in later life.

P1-109

Socioeconomic Differences in Birth Weight and in Maternal Life Style Determinants of Birth Weight Pauline W. Jansen, Henning Tiemeier, Ernst-Jan Troe, Albert Hofman, Vincent W.V. Jaddoe, Johan P. Mackenbach, Rosalinde J.M. Snijders, Frank C. Verhulst and Hein Raat; The Generation R Study Group, Departments of Child and Adoslescent Psychiatry, Epidemiology and Biostatistics and of Public Health, Erasmus MC, Rotterdam, The Netherlands.

Background: Lower maternal socioeconomic status (SES) is associated with adverse pregnancy outcome. It is also known that maternal lifestyle is associated with lower birth weight of the mother's offspring. This raises the question whether maternal lifestyle habits vary with SES. **Methods**: This study is conducted in a subgroup of the Generation R study, a prospective cohort study from early fetal life into adulthood in the Netherlands. Data of 6507 mothers was available for the present study. The educational level of the mother is used as an indicator of SES and is divided in 5 categories. The pregnancy outcome studied is birth weight. Maternal life style determinants of birth weight included smoking, the use of alcohol and drugs and the body mass index of mother (as an indication of eating and exercising habits), all measured around 12 weeks of pregnancy. **Results**: The distribution of participants on the 5 educational levels is as follows: 716 (primary education), 876, 1649, 1008, 1216 (university degree). A linear regression analysis indicated that infants of the lowest educated mothers were 226 g lighter compared to the highest educated mothers [95% confidence interval (CI): 172 – 279]. At 12 weeks of pregnancy lower educated women are likely to have a BMI of 2,31 kg/m² more than the highest educated women [95% CI: 2,83 – 1,79]. The table reflects the results of the logistic regression analyses regarding the relation between educational level and smoking, drinking alcohol and drug use.

Table 1. Odds ratios for exhibiting life style determinants of birth weight (highest educated mothers compared to lowest educated mothers)

Life style behaviors	N [a]	OR	95% CI
Smoked during the 1st trimester	5348	0,258 *	0,204 – 0,325
Still smoking at 12 weeks of pregnancy	1349	0,141 *	0,087 – 0227
Alcohol use during the 1st trimester	5362	9,267 *	7,412 – 11,588
Still drinking alcohol at 12 weeks of pregnancy	2366	2,929 *	1,950 – 4,399
Drug use during the 1st trimester	5418	0,118 *	0,059 – 0,236
Still using drugs at 12 weeks of pregnancy	178	0,315	0,036 – 2,752
[a] Totals vary because of missing data * p = 0.01			

Conclusions: Maternal life style varies with educational level. The lower educated women are more likely to smoke, to use drugs and to have a higher BMI during the first period of pregnancy compared to higher educated women; they even have a higher risk to continue smoking while knowing they are pregnant. The opposite result is found for drinking alcohol. As expected, this study demonstrates that the children of lower educated women are at risk for low birth weight. It is possible that this risk is caused by the life style behaviors (smoking, drug use and BMI) examined in this study; further studies will evaluate this relation in more detail.

P1-110

Relation of Childhood Socioeconomic Position with Adult Mortality Risk Among South Korean Males Young-Ho Khang, MD, PhD; Department of Preventive Medicine, University of Ulsan College of Medicine, Seoul, Korea

Background: Evidence on the relation of childhood socioeconomic position (SEP) with adult mortality risk is mounting but sparse in regions outside Europe and North America. This study was to examine the relation of childhood socioeconomic position (SEP) with mortality risk in South Korea. **Methods**: First round data of the Korea Labor and Income Panel Study were linked to data on mortality. Childhood SEP indicators were father's education, own education, father's occupational class at age 14, own first occupational class after age 15, birth place, and residence at age 14. Adulthood SEP indicators included current occupational class, family income, perceived economic hardships, and current residence. Results: Mortality differentials according to current occupational class, economic hardship and current residence were statistically significant. Mortality risk tended to increase as household income decreased. For all childhood SEP indicators, inverse relationships between childhood SEP and mortality risk were found. These inverse relationships were attenuated but did not disappear with adjustment for adulthood SEP. **Conclusions**: Both early and later life markers of SEP were related to an increased risk of death in South Korea. Relationships between childhood SEP and all-cause mortality persisted even after adjustment for adult SEP. Future studies need to examine the relation of childhood SEP with cause-specific mortality.

P1-111

Birth Weight, Social Destiny and Adult Survival in an Australian Working-Class Population Born between 1857-1900 Janet McCalman, Gita Mishra, Ruth Morley; School of Population Health, University of Melbourne, MRC National Survey of Health and Development, University College London, University of Melbourne Department of Paediatrics and Murdoch Childrens Institute, Australia

Background This project is the first cradle-to-grave study of an Australian population sample. **Methods** Birth weights and maternal characteristics, for births between 1857 and 1900 in a charity hospital in Melbourne Australia, (now the Royal Women's Hospital), were recorded from preserved birth ledgers. Names were linked to death certificates to determine age at death, as well as cause of death.
Results There were16,276 registered live births and death certificates were traced for 8588 (53%). Of these 4300 died in infancy (before 12 months, 50%), and 941 between the ages of one and sixteen years (11%). The remaining 3347 (39%) survived into adulthood (at least 16 years of age), 2958 of them to age 40 or more years (1572 men and 1366 women). End-of-life social location, serving as a proxy for life-long income and accumulated equity, was the most predictive for adult lifespan of all factors analysed. Marriage and fertility were predictive of life span for both men and women. Birth weight was predictive of infant mortality, but not indicative of age of death after the age of sixteen. Historical period of birth was more predictive of infant than adult survival time within this working-class population. Socio-economic status (SES) at birth was strongly predictive of infant mortality, but weakly predictive of male adult lifespan only, suggesting that female lifespan was affected by SES in marriage. Income security and family stability underlay the most significant differences in survival, for both infants and adults. **Conclusions** Life course experience of chronic underemployment and irregular earnings were associated with high accumulation of insults and reduced capacity to recover from economic and personal crises. Biological factors measurable at birth, such as birth weight and parents' SES, were of diminishing significance over the life cycle. This suggests that life-course analysis in historical context may be more revealing for adult health in this colonial-settler working-class population.

P1-112

Maternal Adversity and Maternal Responsiveness: An fMRI Study N.Popeski, C. Scherling, J. Pruessner, & M. Meaney. Douglas Hospital Research Center, McGill University, Montreal, Canada.

Background: Infant cues as well as the mother's emotional state are crucial for directing maternal responsiveness. Infant cues also influence the quality of mother-infant interaction. The quality of the maternal environment has been shown to influence the emotional well-being of the mother and her responsivity to her child. Brain structures that contribute to maternal responsivity in humans remain to be elucidated. **Method**:Thirty-seven subjects (mean age: 29.78) were mothers recruited from an ongoing study (MAVAN). Maternal adversity and responsiveness was assessed through standardized questionnaire tests. We examined the neural correlates of maternal responsivity using functional magnetic resonance imaging (fMRI) in which mothers viewed pictures of their own infant and an unknown infant with either a happy or neutral facial expression. **Results**: Mothers with high levels of maternal adversity had significantly higher trait anxiety and were more depressed than mothers with low levels of adversity. In addition, mothers with more adversity had elevated levels of cortisol during the fMRI scan. We also propose that mothers with high levels of maternal adversity will have less activation within brain regions that are associated with maternal care in response to infant cues. This is the first study linking emotional well-being of the mother with functional neuroimaging in the presence or absence of maternal adversity. (support:CIHR and FRSQ)

P1-113

Socioeconomical Status and Lipid Profile as Risk Factors for Cardiovascular Diseases Liseti Solano, Emma Velásquez, María Adela Barón, María Páez, Daisy Llovera, Zulay Portillo. Centro de Investigaciones en Nutrición. (CEINUT) "Dr. Eleazar Lara Pantin". Facultad de Ciencias de la Salud. Universidad de Carabobo. Valencia, Estado Carabobo. Venezuela. 2001. lsolano@uc.edu.ve

Background: Epidemiological studies have shown that unfavorable serum lipids levels in childhood are predictors of development of atherosclerosis lesions and cardiovascular diseases in adulthood. **Purpose**: In order to evaluate cardiovascular risk factors, 297 Venezuelan preschool children (4-7 years old) from two different socioeconomic levels (high socioeconomic status (HSES) n=103; and low socioeconomic status (LSES), n=194, were assessed. **Methods**: Socioeconomic conditions were determined by modified Graffar method. Nutritional anthropometrical evaluation was performed by weight to height, and NCHS/OMS cut-off point was used. Lipid profile was determined by colorimetric biochemical methods and atherogenic risks factors were calculated. **Results**: Underweight prevalence for HSES group was 5.8% and for LSES: 14.9%, while normal status was 78.6% and 70.1%, and overweight was 15.5% and 14.9%, respectively.

Lipid profile by socioeconomical status was:

Variable	HSES group	LSES group	Statistical test
Triglycerides (mg/dL)	59.0±24.3	67.6±28.1	U: 8134 p< 0.05
Cholesterol (mg/dL)	141.0±25.5	115.6±27.9	U: 4666 p< 0.05
HDL-cholesterol (mg/dL)	40.6±7.1	24.1±6.2	U: 957 p<0.01
LDL-cholesterol (mg dL)	88±23.9	78.0±27.5	U: 7662 p< 0.05
Ratio Col/HDL	3.5±0.78	5.0±1.5	U: 4539 p<0.01
Ratio LDL/HDL	2.0±0.71	3.4±1.4	U: 3822 p<0.01
HDL-cholesterol (<45 mg/dL)	52.4%	97.9%	Chi^2: 95.6; p:0.00
LDL-cholesterol (>110 mg/dL)	6.79%	10.82%	Chi2: 6.64; p:0.03
Ratio col/HDL (>5.0)	3.88%	42.26%	Fisher p<0.001. OR: 18.1
Ratio LDL/HDL (>3.5)	2.91%	42.26%	Fisher p<0.001. OR: 24.4

A significant association was found between lipid values and socioeconomic status, being the LSES preschoolers those with the higher atherogenic risk. Its pattern was of higher triglycerides, lower cholesterol, lower HDL-C levels, and higher Col/HDL and LDL/HDL ratio. Comparisons of lipid profile by nutritional status or gender did not show significant differences. Conclusions: Findings indicate that children from low socioeconomic status are at a higher risk for cardiovascular disease and atherosclerosis than children from high socioeconomic status. Key words: lipid profile, preschoolers, socioeconomic status, cardiovascular risk. Funds: International Development Research Centre (IDRC), Canada and Consejo de Desarrollo Científico y Humanístico (CDCH) – Universidad de Carabobo. Venezuela.

P1-114

The Mind, Body and Time to Pregnancy: Findings from a Random Sample of First Time Mothers Suzanne C. Tough, Karen M. Tofflemire; *Department of Pediatrics and Community Health Sciences, University of Calgary, Calgary, Alberta, Canada.*

Background: It has been noted in developed countries that between 10-15% of all couples will experience infertility at some time during their reproductive lives. Infertility is defined as 12 months of attempted conception without success. After considering male subfertility, ovulation disturbances, defects in spermatozoa-cervical mucus interaction and tuboperitoneal disorders, a significant amount of subfertility remains unexplained (estimated between 15-30%). **Objective**: To examine the characteristics of women who conceived within 12 months of trying compared to those who took longer than 12 months. **Methods:** A computer assisted telephone interview survey of 809 women was conducted. Women were randomly selected from those who delivered their first child, in two major urban areas (Edmonton and Calgary, AB), over 18 months. Data were analyzed using SPSS/PC version 12.0. Bivariate analyses included Odds ratios and Chi Square tests. Subsequent logistic regression, using the forward selection method was used to evaluate the role of physiologic, demographic, and psychologic variables on time to pregnancy using adjusted Odds Ratios (OR) and 95% confidence intervals (95% CI). **Results:** After examining clinically relevant, and other variables cited in the literature, women who took longer than 12 months to conceive were: older (age 35+ OR=3.32 95% CI,1.85-5.97), experienced a tuboperitoneal disorder, such as endometriosis, or had an ovulatory disturbance (OR=4.03, 95% CI, 1.79-9.04) and experienced a previous miscarriage (OR=3.21, 95% CI, 1.88-5.49). Psychologic variables that were related to delayed conception included reporting poor emotional health over the 2 years prior to pregnancy (OR=2.43, 95% CI, 1.40-4.20) and not having experienced a memorable life event (OR=3.34, 95% CI, 1.95-5.72). The most common life events that occurred within 12 months of conception were a marriage (35%), death in the family (21%) and a move (15%). Of note, 65.3% of women taking longer than 12 months to conceive used assisted reproductive technology (medication and/or in vitro fertilization). **Conclusions/Discussion**: Women most likely to be unable to conceive within 12 months can be identified by advanced maternal age, tuboperitoneal disorders, ovulatory disturbances and prior miscarriage. However, other important covariates of delayed conception include poor mental health and the absence of a life event which may trigger decision making toward proactive conception strategies (i.e., increased frequency of intercourse). These findings support the investigation of physiological disturbances when considering the time required for conception and also draw attention to the potential impact of emotional health and salient life events as potential influencers of proactive pregnancy practices.

POSTER SESSION II

Cardiovascular Outcomes

P2-001

Explanations for the Associations of Parental Birthweight with the Birth Weight in Their Offspring Lamise Ay, Vincent W.V. Jaddoe, Albert Hofman, Anita C.S. Hokken, Rosalinde J.M. Snijders, Eric A.P. Steegers and Jaqueline C.M. Witteman; The Generation R Study group, Department of Epidemiology and Biostatistics, Division of Pediatric, Department of Gynaecology & Obstetrics, Erasmus Medical Center, Rotterdam, The Netherlands

Background: Birth weight of parents is associated with birth weight of their offspring. However, it is not known whether other parental risk factors for low birth weight explain this association. **Methods:** This study was embedded in the Generation R study, a prospective cohort study from early fetal life into adulthood in the Netherlands. Data for the present study were available for 6507 mothers, 4764 fathers and 5979 children. First, the association between parental birth weight and birth weight in the offspring was studied. Secondly, we examined whether weight, height, Body Mass Index (BMI), blood pressure, smoking, highest educational level and ethnic background of the parents explained the association. **Results:** As expected, we found a strong association between parental birth weight and birth weight in offspring. Risk factors for low birth weight i.e. height, weight, BMI, smoking, highest educational level and ethnic background were associated with both parental birth weights and birth weight in the offspring. Blood pressure was not associated with parental birth weight nor with birth weight in the offspring. The Table presents the association of both maternal and paternal birth weight on the birth weight in the offspring. After adjustment for all factors together, the association of birth weight of parent and offspring remained significant.

Table Differences in Birth weight categories as compared to reference group

Birth weight	Unadjusted	Adjusted for					
	Mother						
		height	weight	BMI	smoking	Ethnic background	education
2	54 (-17,127)	30 (-17,127)	26 (-48,99)	37 (-67,105)	52 (-22,122)	41 (-30, 114)	49 (-22,122)
3	233 (168,297)	173 (168,297)	183 (117,250)	210 (114,266)	232 (152,281)	210 (145,275)	217 (152,281)
4	419 (334,505)	328 (334,505)	350 (241,418)	374 (259,462)	414 (319,490)	395 (309, 480)	405 (319,490)
	Father						
2	64 (-9,138)	75 (-59,157)	83 (1,165)	84 (12,166)	58 (-16,133)	48 (-28, 125)	28 (-61,118)
3	217 (152,282)	190 (118,262)	213 (141,285)	228 (156,301)	207 (140, 274)	184 (116, 253)	181 (101,260)
4	355 (278,432)	307 (223,391)	333 (249,417)	362 (278,446)	342 (263,420)	324 (244,404)	301 (210,392)

Values are means (Birth weight category (gram):1) <2500 (reference category) 2)2500-2999 3)3000-3999 4) >= 4000

Conclusion: Birth weight of both mother and father is strongly associated with birth weight of the offspring. Height, weight, BMI, highest educational level, ethnic background and smoking of mother and father all explain part of the association between birth weight of the parent and the child. However these factors combined do not fully explain the association.

P2-002

Age Independent Association Between Low Birth Weight And All-Cause Mortality In Adulthood Among 199,639 Danish Men And Women Jennifer L. Baker, Lina W. Olsen, Thorkild I.A. Sørensen. Institute of Preventive Medicine, Copenhagen University Hospital, Øster Søgade 18, 1399 Copenhagen K, Denmark

Background: Although low birth weight is consistently associated with cardiovascular disease in adulthood, its association with all-cause mortality is not well studied. Therefore, we investigated if birth weight is associated with all-cause mortality in adulthood. **Methods:** Our subjects were 199,639 men and women who attended school in Copenhagen, Denmark and were born from 1936 to 1979. Birth weight was treated as a continuous variable and divided into 4 categories: 2000 - 2750, 2751 - 3250, 3251 - 3750, 3751 - 6000 g. We created a birth cohort variable by dividing the year of birth into 4 intervals: 1936 - 1940, 1941 - 1950, 1951 - 1960, 1961 - 1979. All analyses were performed separately for each sex and stratified by birth cohort. Proportional hazards assumptions were investigated with Nelson-Aalen plots and Cox regressions were performed. Age was the underlying time variable. **Results:** Among the 102,533 men, 10,644 deaths occurred during 2,539,213 person-years of follow-up. Among the 97,106 women, 6259 deaths occurred during 2,431,052 person-years of follow-up. The Nelson-Aalen plots showed that, for one birth weight category compared to another, the relative risk (RR) of death among men and women was the same for all ages from 25 - 70 years. Therefore, the proportional hazards assumptions were fulfilled, and a Cox regression could be performed. Compared to men who had a birth weight between 3251 - 3750 g, those in the lowest category of 2000 - 2750 g had a 1.14 increased RR (95% CI: 1.07 - 1.22) of all-cause mortality. Compared to women who had a birth weight between 3251 - 3750 g, those in the lowest category of 2000 - 2750 g had a 1.24 increased RR (95% CI: 1.14 - 1.33) of all-cause mortality. **Conclusions:** In this population, we found an age-independent association between low birth weight and all-cause mortality among men and women. At every age, from 25 - 70 years, the 14% increased risk among men and the 24% increased risk among women of all-cause mortality associated with low birth weight remained constant. The remarkable stability of this association suggests that low birth weight is an indicator for an increased risk of impaired health throughout adult life.

P2-003

Hypertension, Glomerular Number, and Birth Weight in African Americans and Whites in the Southeastern United States Michael D. Hughson[1], Rebecca Douglas-Denton[2], John F. Bertram[2], Wendy E. Hoy[3]; *[1]Department of Patholgy, Uiversity of Mississippi Medical Center, Jackson, USA. [2]Department of Anatomy and Cell Biology, Monash University, Clayton, Victoria, Australia. [3]Centre for Chronic Disease, The University of Queensland, Brisbane, Australia.*

Background: Low nephron number has been related to low birth weight and hypertension. In the Southeastern United States, the estimated prevalence of chronic kidney disease due to hypertension is five times greater for African Americans than whites. This study investigates the relationships between total glomerular number (N_{glom}), blood pressure, and birth weight in Southeastern African Americans and whites. **Methods:** Stereological estimates of N_{glom} were obtained using the physical dissector/fractionator technique on autopsy kidneys from 62 African Americans and 60 whites 30 to 65 years of age. By medical history and recorded blood pressures 41 African Americans and 24 whites were identified as hypertensive and 21 African Americans and 36 whites as normotensive. Mean arterial blood pressure (MAP) was obtained on 81 and birth weights on 63 subjects. **Results:** For African Americans, relationships between MAP, N_{glom}, and birth weight were not significant but they were for whites: MAP and N_{glom} (r=-0.4551, P=0.0047); N_{glom} and birth weight (r=0.5730, P=0.0022); MAP and birth weight (r=-0.4228, P=0.0377). For African Americans, average N_{glom} of 961,840 ± 292,750 for normotensive and 867,358 ± 341,958 were not significantly different (P=0.285). For whites, average N_{glom} of 923,377 ± 256,391 for normotensive and 754,319 ± 329,506 for hypertensive patients were significantly different (P=0.03). **Conclusions:** The data indicate that low nephron number and possibly low birth weight may play a role in the development of hypertension in whites but not African Americans

P2-004

Gestational Iron-Deficiency Results in Cardiac and Renal Hypertrophy Stephane L Bourque, Corry Smallgange, Michael A Adams, Kanji Nakatsu. *Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.*

Introduction: The World Health Organization estimates that 4-5 billion people worldwide (66-80% of the global population) are iron deficient (WHO; 2003); the populations most at risk in both industrialized and developing countries are pregnant women and those of childbearing ages. Importantly, maternal iron-deficiency during pregnancy has been shown to induce fetal programming effects, which render it more prone to develop a number of diseases in later life, including hypertension. The purpose of this study was to determine the impact of severe maternal iron-deficiency on organ growth and development of the offspring. **Methods:** Adult female Wistar rats were placed on either a high iron purified diet (225ppm iron) or a low-iron purified diet (3ppm iron) for two weeks prior to conception. Females were subsequently mated with control-fed male Wistar rats. At 24 hours following birth, body weights, tissue weights and hematocrit values were assessed. **Results:** Offspring from iron deficient mothers had significantly reduced mean hematocrit values (25.5 ± 3.8% versus 41.1 ± 4.0 %) and body weights (5.35 ± 0.54g versus 5.87± 0.65g) compared to control groups. Neonatal heart weights in these offspring were significantly increased (6.67 ± 0.51 mg/g body weight) compared to controls (5.12 ± 0.47 mg/g body weight). Interestingly, kidney weights relative to body weight were also found to be significantly elevated in the iron deficient group (5.33 ± 0.56 mg/g) compared to controls (4.78 ± 0.46 mg/g). **Summary:** These results suggest an important role for iron in the growth and development of numerous organs, particularly those involved in the establishment and regulation of blood pressure.

P2-005

Elevated Endothelin-1 and Hypertension in Adult Offspring of Intrauterine Growth Restricted Newborns Mina Desai, Omid Khorram, Dave Gayle, and Michael G. Ross Department of Obstetrics and Gynecology, David Geffen School of Medicine at UCLA and LABioMed at Harbor-UCLA Medical Center, Torrance, CA 90502, USA

Bacvkground: Human and animal studies have demonstrated the gestational programming of offspring hypertension. Although thought to be secondary to perintal stress, the mechanism(s) involved in the development of offspring hypertension has not been elucidated. The endothelin system has been demonstrated to participate pathophysiologically in numerous conditions, including hypertension, atherosclerosis, coronary artery disease, heart failure and diabetes. Endothelial injury is the main stimulus for endothelin-1 (ET-1) secretion - a potent vasoconstrictor. As ET-1 is also a potent growth-promoting agent, the enhanced ET-1 production in some hypertensive models contributes to the remodeling of large and small arteries. We sought to determine whether ET-1 levels are associated with gestational programming of offspring hypertension. **Methods:** Pregnant Sprague Dawley rats and offspring were

studied. Control dams (n=6) received ad libitum food, whereas study dams (n=6) were 50% food-restricted from pregnancy day 10 to 21 to produce IUGR newborns. At birth, litter size was culled to 4 males and 4 females. All pups were nursed by dams fed ad libitum and were weaned at 3 weeks to ad libitum feed. At age 3 months, systolic blood pressure was measured using tail-cuff method (ML125 NIPB System, ADInstruments). At age 9 months, ET-1 was determined. Values shown are mean±SE from male offspring. **Results:** Though small at birth (6.0±0.3 vs 7.1±0.3 g, $p<0.01$), at 3 months of age IUGR offspring were heavier (498±10 vs 403±9 g, $p<0.01$) and had increased systolic blood pressure (152±3 vs 146±2 mmHg, $p<0.05$) as compared to the controls. At 9 months of age, IUGR offspring were markedly heavier (742±15 vs 647±18 g, $p<0.001$) and had significantly elevated plasma ET-1 levels (166±20 vs 82±17 pg/ml, $p<0.01$). **Conclusion:** Maternal nutrient restriction results in offspring with significant hypertension, potentially secondary to vasoconstrictive actions of ET-1. We speculate that endothelin antagonists may prevent or treat gestationally programmed hypertension.

P2-006

Does Adjusting for Body Size Affect the Relation Between Birthweight and Hypertension? [1]George TH Ellison, [2,3]Yu-Kang Tu, [2]Robert West, and [2]Mark S Gilthorpe; [1]St George's, University of London, Cranmer Terrace, London SW17 0RE; [2]Biostatistics Unit, Centre for Epidemiology and Biostatistics, University of Leeds, Leeds LS2 9LN; [3]Leeds Dental Institute, University of Leeds, Leeds LS2 9LU.

Background: Computer simulations explored the role of the 'reversal paradox' on the impact of adjusting for one or more measure of adult body size on the relation between birthweight (*BW*) and adult systolic blood pressure (*SBP*) **Methods:** Assuming that there is no statistical relation between *BW* and *SBP* (i.e. that for the bivariate correlation Pearson's $r = 0.0$), the function 'mvrnorm' in the MASS package in *R* was used to generate multivariate normal data on a hypothetical sample of 500 30-year-old males for: *BW*; *SBP*; current weight (*CW*); current body mass index (*CBMI*); and current height (*CH*); based on mean values, standard deviations, and correlations drawn from the literature. Three models were generated after adjusting for one (*CW*), two (*CW* and *CBMI*), or three (*CW*, *CBMI* and *CW*) measures of current body size. To simplify the analyses, the simulations assumed that the three measures of adult body size all had the same correlation with both *BW* and *BP*, and three sets of models were developed in which these correlations varied from a Pearson's correlation of $r = 0.1$, 0.2 to 0.3. **Results:** Even though the bivariate correlation between *BW* and *SBP* was set to zero, the simulations demonstrated that the median partial regression coefficient for *SBP* on *BW* became negative after adjusting for current body size. Furthermore, the simulations demonstrated that the strength of the coefficient increased (i) after adjusting for additional measures of current body size; and (ii) as the correlation between these measures of body size and both *BW* and *SBP* increased from $r = 0.1$ to 0.3 (see Table 1).

Table 1: Median partial regression coefficients of *SBP* on *BW* after adjusting for three measures of current body size, when the unadjusted bivariate correlation between *BW* and *SBP* is 0.

	BW		CW		CBMI		CH	
	median	95% CI	median	95% CI	median	95% CI	median	95% CI
BP – current body size correlations and BW – current body size correlations = 0.10								
Model 1	-0.50	-2.33, 1.42	0.12	0.05, 0.19				
Model 2	-0.90	-2.70, 1.05	0.10	0.04, 0.17	0.37	0.13, 0.60		
Model 3	-1.17	-3.04, 0.69	0.09	0.02, 0.17	0.33	0.08, 0.56	0.22	0.07, 0.38
BP – current body size correlations and BW – current body size correlations = 0.20								
Model 1	-0.84	-2.77, 1.03	0.16	0.10, 0.22				
Model 2	-1.48	-3.37, 0.35	0.14	0.07, 0.21	0.49	0.24, 0.73		
Model 3	-1.99	-3.81, -0.14	0.12	0.06, 0.19	0.43	0.18, 0.66	0.28	0.13, 0.44
BP – current body size correlations and BW – current body size correlations = 0.30								
Model 1	-2.17	-4.02, -0.35	0.25	0.19, 0.32				
Model 2	-3.46	-5.35, -1.59	0.21	0.14, 0.27	0.72	0.50, 0.96		
Model 3	-4.37	-6.17, -2.53	0.17	0.11, 0.24	0.61	0.40, 0.86	0.41	0.24, 0.56

Conclusion: These findings suggest that the impact of the 'reversal paradox' on the association between birthweight and blood pressure is exacerbated by adjusting for more than one measure of current body size. A similar trend has been observed amongst empirical studies reporting both unadjusted and body size-adjusted associations between birthweight and blood pressure.

P2-007

Early Growth of Children from a Bilingual Area in Finland and their Risk of Coronary Heart Disease T FORSÉN[1], E KAJANTIE[1], C OSMOND[3], DJP BARKER[3], JG ERIKSSON[1 1)]National Public Health Institute, Helsinki, Finland. [2)]MRC Epidemiology Resource Centre, UK, University of Southampton, UK. [3)]Developmental Origins of Health and Disease Division, UK

Background: Finland is a bilingual country with Finnish and Swedish as official languages. The Swedish-speaking minority in western Finland has a lower cardiovascular mortality than the Finnish-speaking majority from the same region. The difference in life expectancy is not explained by conventional risk factors. **Aims:** To study whether differences in mortality from coronary heart disease between the language groups could be explained by differences in early growth. **Methods:** We studied 13445 children (11.8% Swedish-speaking) from two cohorts born in Helsinki during 1934-44 whose growth during childhood was recorded. We identified 275 children who had died from coronary heart disease between 1971-2003. **Results:** There was no difference in body size at birth between the language groups ($p=0.2$ for birth weight and $p=0.5$ for length). The Swedish-speaking children increased their growth rate in infancy and they gained both weight and height more rapidly between 6 months and 3 years of age ($p<0.0001$). They were significantly taller thereafter. After the age of 3 their growth rate was similar to the other children. The Swedish-speaking children had significantly lower mortality from coronary heart disease (OR=0.65 95% CI 0.43-0.98). **Conclusions:** The Swedish-speaking children had a higher rate of growth from 6 months up to the age of 3 years and they were significantly taller. The lower mortality from coronary heart disease in the Swedish-speaking children may be due to a more optimal early growth.

P2-008

What is the Role of Adult Height in the Relation Between Birthweight and Blood Pressure? A Systematic Review [1]Rosemary Head, [1]George T.H. Ellison, [2]Mark S. Gilthorpe; St George's – University of London, Cranmer Terrace, London SW17 0RE; [2]Biostatistics Unit, Centre for Epidemiology and Biostatistics, University of Leeds, Leeds LS2 9LN, United Kingdom

Background: Much has been made of the possible utility of catch-up growth to differentiate between individuals who were small at birth as a result of undernutrition *in utero*, and those who were small at birth as a result of a lower genetic potential. Numerous studies have approached this issue by controlling for body weight or body mass index, but these markers of attained size are flexible and respond to short term changes in energy balance. For this reason, adult height is likely to be a better marker of growth potential, albeit for those individuals whose postnatal growth was unconstrained: because it is impossible to determine whether short individuals were undernourished in childhood or have a lower genetic potential for growth, height only provides an unequivocal measure of growth potential for tall individuals. **Methods:** A systematic review of the literature was conducted to examine the role of height in the relationship between birthweight and blood pressure, using a search of Medline based on Huxley *et al.* (2000) extended to include articles published up to 2004. Articles were included if: blood pressure or hypertension was an outcome variable; birthweight and height were exposure variables; and study participants were at least 25 years old. Studies were excluded if they contained no statistical analyses of the relation between birthweight and blood pressure, or if they were studies of particular groups with pre-existing clinical conditions. **Results:** Only three published studies contained analyses that combined measurements of birthweight, adult height and adult blood pressure (Berkey *et al.*, 1998; Langenberg *et al.*, 2003; and Koupilova *et al.*, 1997). The first of these, which sought to identify anthropometric predictors of blood pressure amongst 134 30-year-old individuals from Boston, found no significant relationship between height and either systolic (r=0.04; p>0.05) or diastolic (r=0.10; p>0.05) blood pressure, and found that weight and body mass index were better predictors of blood pressure (Berkey et al., 1998). The second study, which compared height, leg length and trunk length as predictors of blood pressure amongst 2,718 members of the 1946 birth cohort, found a significant decrease in systolic (but not diastolic) blood pressure with increasing height, and that this only remained significant for the tallest quartile after controlling for birthweight (Langenberg *et al.*, 2003). The last study, which examined the relation between birthweight and hypertension in 686 Swedish men aged 50, 60 and 70, found an interaction with height such that birthweight was inversely associated with hypertension only in those above median height (>176m; Koupilova *et al.*, 1997). **Conclusions:** As expected, height was a heterogeneous measure of undernutrition in early life and was poorly correlated with blood pressure. Moreover, the tendency for blood pressure to decrease with increasing height was largely confounded by birthweight. Nonetheless, the interaction between height and blood pressure confirms that height is a powerful marker of growth potential amongst tall individuals, and is a crucial variable for exploring the developmental origins of adult disease. **References:** Berkey *et al.* (1998) *Obesity Research* **6**:187-95; Huxley *et al.* (2000) *Journal of Hypertension* **18**:815-31; Koupilova *et al.* (1997) *Blood Pressure* **6**:223-8; Langenberg *et al.* (2003) *Journal of Hypertension* 21:537-43.

P2-009

Risk of High Blood Pressure Among Young Men Increases with Degree of Immaturity at Birth Stefan Johansson, MD, Anastasia Iliadou, MSc, PhD, Niklas Bergvall, MSc, Torsten Tuvemo, MD, PhD,Mikael Norman, MD, PhD, Sven Cnattingius, MD, PhD; *Department of Medical Epidemiology and Biostatistics, Karolinska institutet, PO Box 281, SE - 171 77 Stockholm, Sweden*

Background Survivors of preterm birth constitute a new generation of young adults, but little is known about their long-term health. We investigated the association between gestational age and risk of high blood pressure in young Swedish men, and whether gestational age modified the risk of high blood pressure related to being born small for gestational age (SGA). **Methods** Population-based cohort study including 329,495 Swedish men, born 1973-1981 and conscripted for military service 1993-2001. Main outcome measures were high systolic and diastolic blood pressures at conscription. **Results** Compared to men born at term (gestational age 37-41 weeks), the adjusted ORs (95% CI) for high systolic blood pressure (= 140 mm Hg) were as follows: moderately preterm (33-36 weeks) 1.24 (1.19-1.30); very preterm (29-32 weeks) 1.48 (1.30-1.68); and extremely preterm (24-28 weeks) 1.93 (1.34-2.76). Being SGA was only associated with increased risk of high systolic blood pressure among men born at 33 weeks or later. The risk estimates for high diastolic blood pressure (=90 mm Hg) increased with decreasing gestational age, and the trend across the gestational age categories was highly significant (p for trend < 0.001). **Conclusions** Preterm birth, a common pregnancy complication, is a strong risk factor for high blood pressure in young men. The risk of high systolic blood pressure associated with birth weight for gestational age is modified by gestational age, suggesting that perinatal contributions to blood pressure elevation later in life may be induced by different biological pathways.

P2-010

Gender Specificity of Prenatal Influences on Cardiovascular Control During Stress in Prepubertal Children: Multiple Pathways to the Same Disease Endpoint? Alexander Jones,[1] Alessandro Beda,[2] Clive Osmond,[1] Keith M. Godfrey,[1] David M. Simpson[2] and David I. W. Phillips[1]; [1]*MRC Epidemiology Resource Centre, University of Southampton, UK.* [2]*Institute of Sound and Vibration Research, University of Southampton, UK.*

Background: Adverse fetal environments, reflected by small size at birth, are associated with later hypertension and cardiovascular disease. Prenatal adaptations of autonomic cardiovascular control which persist into later life may underlie this observation and may be gender-specific. Although this is supported by animal experiments, little data in humans exists and is exclusively from adults. Therefore, studies of prepubertal children are needed to investigate the relationship between prenatal development and later cardiovascular control, prior to the potential confounding influences of gonadal hormones and the development of cardiovascular disease. **Aim**: To investigate whether smaller but otherwise healthy term babies exhibit altered cardiovascular control during psychological stress in childhood.
Methods: We carried out a cross-sectional study of 68 boys and 72 girls (aged 7-9 years) who have been followed since 12 weeks of gestation when their mothers took part in a study of healthy children born in Southampton, United Kingdom. Cardiovascular function was assessed using continuous blood pressure, ECG and impedance cardiography measurement during a restful task and whilst the children underwent a psychological stress test (Trier Social Stress Test for Children). This allowed us to derive measures of heart rate, pre-ejection period (a marker of cardiac sympathetic activation), stroke volume and cardiac output which were used to calculate systemic vascular resistance, and corrected QT interval (a marker of cardiac repolarisation rate which is related to cardiac sympathetic activation). **Results**: In boys, markers of fetal growth restriction, such as low birthweight, were associated with increased arterial pressure ($r = -0.62$, $P < 0.01$) and increased systemic vascular resistance ($r = -0.47$, $P < 0.05$), particularly following the stress test. In contrast, girls who were small at birth showed no such associations, but did show greater cardiac sympathetic nervous system activation as indicated by measures of pre-ejection period ($r = 0.53$, $P < 0.01$) and corrected QT interval ($r = 0.45$, $P < 0.01$) both at rest and during stress. These associations were independent of gestational age and obesity.
Conclusions: Our findings suggest that processes occurring during fetal life, that result in a smaller newborn, have lasting effects on cardiovascular function which differ by gender. They suggest that girls who were small at birth exhibit greater ß-adrenergic activation of the heart, whilst smaller boys exhibit greater a-adrenergic mediated vasoconstriction in response to a stressful environment. Furthermore, boys, but not girls, showed an association between small size at birth and increased blood pressure. We recently found associations between small size at birth and greater sympathetic activation, reduced parasympathetic activation and reduced baroreflex control in young adult women but not men. These women also had raised blood pressure suggesting that although altered autonomic nervous system function is present in childhood in females, associated blood pressure changes do not appear until adulthood. There is now evidence from human studies that cardiovascular responsiveness to psychological stress predicts later development of hypertension. Our study suggests that individuals who were small at birth may arrive at this disease endpoint through prenatal influences on different cardiovascular control mechanisms.

P2-011

Prevalence of High Diastolic Blood Pressure Among Young Rural Indian Adults Shobha Rao, Asawari N. Kanade, Kanchankumar Patil and Sayali Deshpande. Biometry & Nutrition Group, Agharkar Research Institute, G.G. Agarkar Road, Pune 411 004, India.

Background: Blood Pressure is known to be the link between intrauterine or early postnatal environment and adult cardiovascular diseases. Measurements at birth have shown better predictors of blood pressure. Especially Diastolic Blood Pressure (DBP) is seen to be increased among growth retarded fetuses & chance of persistence of high DBP at later ages was more among children who were thin than obese children during early years of life. **Methods:** Cohort of young (19-26 yr) rural adults (Males =310; Females =170) was measured currently for anthropometry (Weight, height, body circumferences and skinfold thicknesses) and blood pressure (Systolic and Diastolic) using standard equipments, for whom the anthropometric measurements were also available during early childhood (0-4 yr). **Results:** Prevalence of high DBP(>=80mmHg, 40.4%) was significantly ($p<0.01$) higher than that of high SBP(>=130mmHg, 21.0%). The prevalence of high DBP was significantly ($p<0.01$) higher in males (45.8%) than in females (30.6%). While examining the association of DBP among males who were stunted in preschool age(0-4yr), it's prevalence was significantly ($p<0.01$) higher in males (41.8%) than in females (25.0%). Further, this prevalence among males increased with increasing adult BMI (68.4%) or Waist circumference (68.8%) but short as adults. These findings suggest that the risk for high diastolic blood pressure is elevated among those who were stunted in early life and remained short as adults but have higher current BMI and waist circumference. Probably this might be the reflection of increased vascular resistance among these chronically undernourished rural young adults.

P2-012

Effects of an Antiatherogenic Diet During Pregnancy on Markers of Maternal and Fetal Endothelial Activation and Inflammation Janette Khoury, Tore Henriksen, Ingebjørg Seljeflot, Lars Mørkrid, Kathrine Frey Frøslie, Serena Tonstad, Department of Obstetrics and Gynecology, National University Hospital, Oslo, Norway and Department of Preventive Cardiology Ullevaal University Hospital, Oslo, Norway , Department of Obstetrics and Gynecology, NationalUniversity Hospital, Oslo, Norway, Center for Clinical Research Ullvaal University Hospital, , Department of Clinical Chemistry, National University Hospital, Oslo, Norway, Department of Biostastistics National University Hospital, Oslo, Norway, Department of Preventive Cardiology Ullevaal University Hospital, Oslo, Norway.

Background. The Cardiovascular Risk Reduction Diet in Pregnancy (CARRDIP) trial showed that a cholesterol lowering diet reduced maternal plasma levels of atherogenic lipids. Moreover, the diet was associated with a marked reduction in the incidence of preterm delivery. Inflammatory mechanisms are involved in atherogenic processes as well as in several pregnancy complications including preterm delivery. In non-pregnant individuals dietary intervention has been shown to influence inflammatory parameters and other novel cardiovascular risk markers. **Aim** . To study the effect of an antiatherogenic diet in pregnancy on maternal inflammatory parameters, we measured maternal and cord blood levels of a range of markers of endothelial activation, inflammation and thrombosis. **Methods.** CARRDIP was a randomized, single blinded trial conducted among nonsmoking women aged 21 to 38 years carrying a single fetus and with no previous pregnancy-related complications. Women in the intervention group (N=141) were advised to consume fish, low-fat meats, oils and low-fat dairy products instead of full-fat dairy products and meats and to eat more whole grains, fruits, vegetables and legumes. Women in the control group (N=149) followed their usual diet. Soluble forms of cellular adhesion molecules (vascular adhesion molecule-1 [VCAM-1], intercellular adhesion molecule-1 [ICAM-1], and E-selectin) were measured at 17-20 weeks of gestation (baseline), and at weeks 30 and 36. High sensitivity CRP [hs CRP] was measured at baseline and subsequently at weeks 24, 30 and 36. Hemostatic parameters (plasminogen activator inhibitor type-1 activity[PAI-1], plasminogen activator inhibitor type-2 antigen [PAI-2], tissue plasminogen activator antigen [tPAag], and von Willebrand factor) were measured at baseline and week 36. All the above, except CRP, were also measured in cord blood. **Results**. Maternal concentrations of all markers of inflammation and endothelial cell activation, except for CRP, increased during the study period in both arms of the study. However, none of the markers in maternal as well as in cord blood were significantly influenced by the intervention Furthermore, when we compared the levels of inflammatory markers in women with and without preterm birth in the whole cohort no differences in levels of the measured inflammatory markers were found except for a higher gestational increase in VCAM-1 from baseline to week 30 in women with preterm birth compared with women who delivered at term, mean difference -66.1 ng/mL [95% CI -111,1. -21.2]; $P < 0.01$. **Conclusion**. An antiatherogenic diet in pregnancy did not influence maternal concentrations of a range of markers of endothelial activation and inflammation, most of which increased during the study period. Thus the previously reported effects of a cholesterol lowering diet during pregnancy on maternal lipid profile and risk of preterm delivery do not seem to involve changes in the systemic inflammatory responses of pregnancy.

P2-013

Mother's Pelvic Size is Related to Circulatory Disease in the Offspring Among Swedes Born 1915-29 Ilona Koupil and David A. Leon; Centre for Health Equity Studies (CHESS), Stockholm University/Karolinska Institute, Stockholm, Sweden, Department of Epidemiology and Population Health, London School of Hygiene & Tropical Medicine, London, UK

Background: Size and shape of the bony pelvis are known determinants of the progress of labour and delivery. Size and shape of mother's pelvis have been also shown to influence birth weight of the male offspring and their risk of stroke but not coronary heart disease. We investigated the associations of mother's pelvic size with the offspring circulatory disease among men and women born in Uppsala in 1915-1929. **Methods**: Interspinal diameter, intercristal diameter and conjugata externa were abstracted from obstetric records for mothers of 7704 men and women whose size at birth and length of gestation was known and who were followed for cause-specific mortality from 1961 till end 2002. Additional data on social characteristics of the study

subjects were obtained from routine registers. **Results**: Measures of pelvic size were highly correlated, associated positively with birth weight and weight for gestational age, but not related to length of gestation. In offspring born from full-terms pregnancies, 1 cm increase in interspinal diameter adjusted for parity was associated with an increase in birth weight of 34g (95% CI 21 to 46). Later age at menarche was associated with smaller pelvic size. Among men and women born 1915-1929 mothers' pelvic size was inversely associated with risk of circulatory disease and stroke in particular. The associations of pelvic size with death from circulatory disease remained significant after adjustment for age, period, birth weight, length of gestation and social characteristics and tended to be stronger in women. Fully adjusted hazard ratio for death from stroke per 1 cm increase in intercristal diameter was 0.82 (95% CI 0.70 to 0.96). **Conclusions**: Mother's pelvic size is predictive of mortality from circulatory disease among people born in the first third of the 20th Century. More research on effects of pelvic size in younger cohorts is needed in order to judge the importance of our findings.

P2-014

Lipid Profile in Middle Age After Food Restriction During Gestation: The Dutch Famine of 1944-45 L.H. Lumey, Aryeh D. Stein; Patricia A. Zybert; Henry S. Kahn; Gerard-Jan Blauw; Karin M. van der Pal – de Bruin. Mailman School of Public Health, Columbia University, New York NY, USA; Department of Global Health, Emory University, Atlanta GA, USA; Centers for Disease Control and Prevention, Atlanta, GA, USA; Leiden University Medical Center, Leiden, the Netherlands; TNO Quality of Life, Leiden, the Netherlands.

Background: Many studies in humans have assessed the inverse association between fetal growth and lipid-related cardiovascular risk factors in later life but few studies have related these observations to specific maternal factors. We used the circumstances of the Dutch Famine of 1944-45 to assess whether generalized reductions of maternal food intakes at specified stages of pregnancy were related to a more atherogenic profile in adult offspring. **Methods**: We recruited three series of subjects: (1) exposed individuals born in one of three institutions in western Holland between January 1945 and March 1946, whose mothers were all exposed to the famine during or immediately preceding pregnancy; (2) unexposed individuals born in the same three institutions during 1943 or 1947, whose mothers did not experience famine exposure during this pregnancy; and (3) unexposed same-sex siblings of subjects in series 1 or 2. Fasting blood samples were obtained from 437 men and 534 women between 2003 and 2005 and were analyzed for total cholesterol, HDL-cholesterol and triglycerides (all reported in mmol/L) with standard methods. We defined four (partially overlapping) windows of gestational exposure (gestational weeks (GW) 1-10; 11-20; 21-30; and 31 through delivery) based on exposure to a ration <900 kcal/day during the whole 10-week interval. Maternal preconception exposure status was characterized by a score representing cumulative weeks of exposure to reduced rations in the six months prior to conception. We assessed the joint effects of exposure in one or more periods of gestation with hierarchical (GEE) regression models to account for within-sibship correlations. We also examined the effect of adjustments for adult height and waist circumference (WC). **Results:** In men, the ratio of total to HDL-cholesterol was lowered after prenatal famine exposure in pregnancy weeks 21-30 and increased after exposure in weeks 11-20 (both at $p < 0.05$). In women, total cholesterol and triglycerides were elevated after exposure in pregnancy weeks 1-10 ($p < 0.10$) (Table). Additional adjustment for height and WC did not affect these patterns.

	Period of exposure to reduced rations Difference from unexposed (# p<0.10; * p<0.05). Units in mmol/L					
	Unexposed	Exposure weeks 31 -delivery	Exposure weeks 21-30	Exposure weeks 11-20	Exposure weeks 1-10	Preconception Exposure
Males						
Total chol	5.47+/-0.95	-0.077	-0.210	0.124	0.083	-0.007
HDL-chol	1.37+/-0.36	-0.011	0.058	-0.046	0.036	-0.032
Triglycerides	1.64+/-0.94	0.062	-0.087	-0.090	0.323	0.033
TOTc/HDLc	4.26+/-1.27	-0.109	-0.544 *	0.492 *	-0.077	0.052
Females						
Total chol	5.79+/-1.09	0.224	-0.130	0.225	0.355 #	-0.044
HDL-chol	1.74+/-0.46	0.060	-0.059	-0.017	-0.060	-0.130
Triglycerides	1.36+/-0.75	0.057	0.211	-0.007	0.226 #	0.158
TOTc/HDLc	3.55+/-1.19	-0.104	0.188	0.118	0.291	0.160

Conclusions: In this population, no consistent relation was seen between nutrition in pregnancy and adult lipid profiles. These results do not strongly support previously reported findings from the Netherlands that maternal malnutrition during early gestation may program lipid metabolism in the offspring.

P2-015

Birth Weight and Coronary Heart Disease in a Cohort Born 1857 - 1900 in Melbourne, Australia Ruth Morley, Janet McCalman, John B Carlin. University of Melbourne Department of Paediatrics, and Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Centre for Health and Society, School of Population Health, University of Melbourne, Australia.

Background The widely observed association between birth size and risk of later coronary heart disease (CHD) has not been examined in a pre-20th century cohort in which many individuals would have been impoverished throughout their life-course. **Methods** Birth weights and maternal characteristics, for births between 1857 and 1900 in a charity hospital, were recorded from preserved ledgers. Names were linked to death certificates to determine age and cause of death. Death with CHD was coded using specific criteria, and survival analysis methods were used to relate risk of CHD to birth weight, allowing for competing causes of death and adjusting for potentially confounding maternal factors. **Results** Death certificates were traced for 8588 (53%) of 16,276 registered live-births. Analyses were confined to 2938 subjects (1572 male, 1366 female) who survived beyond age 40, since none of 486 cases was recorded earlier. Risk of CHD increased with time, but there was no evidence that it was related to birth weight, in men or women.

Table: *Hazard ratios indicating relative risk of death with coronary heart disease according to birth weight and year of birth, from multivariable Cox regression model.*

	MALES (n = 1571)		FEMALES (n = 1366)	
	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Birth weight				
per kg increase	0.9	0.7, 1.1	1.0	0.8, 1.4
Year of birth				
1857-1865	1		1	
1866-1870	2.1	0.6, 7.2	8.8	1.1, 71.7
1871-1875	2.4	0.7, 7.6	7.1	0.9, 58.6
1876-1880	5.2	1.8, 15.5	6.9	0.9, 53.7
1881-1885	4.6	1.5, 13.7	11.5	1.5, 87.5
1886-1890	6.8	2.3, 20.4	9.4	1.2, 73.1
1891-1895	8.8	2.8, 23.1	23.1	3.1, 172.4
1896-1900	8.5	3.0, 24.0	26.6	3.6, 197.8

Conclusions We did not replicate findings in more recent cohorts. This may represent a true lack of association in a historical cohort who generally remained impoverished throughout their life-course. However, we acknowledge the strong possibility of misclassification of cause of death by the person filling in the death certificate and/or our coding criteria. The observed increase in CHD over time could reflect change in prevalence, diagnosis, or both. We failed to trace almost 50% of death certificates, but see no reason why this should relate to birth weight or CHD. Multi-factorial life course analysis, in historical context of time and place, may provide better understanding of these mortality data.

P2-016

Maintenance of Mean Arterial Blood Pressure in Chronically Hypoxic Fetal Sheep is not Dependent on Post Ganglionic Sympathetic Activation J.L. Morrison, I.C. McMillen, Discipline of Physiology, Centre for the Early Origins of Adult Health, University of Adelaide, Adelaide, Australia, 5005.

Background: Epidemiological studies demonstrate an association between intrauterine growth restriction and increased risk of cardiovascular disease in adult life. The mechanisms underlying this association are less well understood. We have shown that placental restriction (PR) in the sheep, resulting from removal of the majority of the endometrial caruncles prior to conception, results in chronically hypoxic, growth restricted fetuses. PR fetuses have higher circulating plasma noradrenaline concentrations than control fetuses. Pharmacologic studies show that the PR fetus exhibits a greater fall in mean arterial pressure (MAP) during α-adrenergic blockade. This study aimed to determine the role of post ganglionic sympathetic activation in regulating MAP, heart rate (HR) and femoral artery blood flow (FBF) in growth restricted fetal sheep. **Methods**: Four ewes underwent carunclectomy surgery 10 wk prior to mating. Fetal catheterisation was performed at 104-118 d gestation (term, 150 d) in 6 control and 4 PR fetuses. At 117.6±0.4 d gestation, fetuses received a bolus infusion of saline followed 1 h later by a bolus i.v. injection of 16.5±2.2 mg/kg hexamethonium bromide, a nicotinic antagonist which blocks post ganglionic sympathetic activation. MAP, HR and FBF were monitored using Chart 4 (PowerLab, ADInstruments, Sydney, AUS) for 2 h before and 24 h after drug treatment. **Results**: PR fetuses had a lower PO_2 (17.5±1.3 mmHg) than control fetuses. There was no difference in the effect of hexamethonium on MAP, HR or FBF in PR and control fetal sheep. **Conclusion**: Thus these data suggest that the increased dependence of maintenance of MAP, HR and femoral artery blood flow on α-adrenergic activation in the PR fetus is not a result of increased post ganglionic sympathetic activation.

	Control (n=6)	PR (n=4)
MAP (mmHg)	-4.3±1.0	-5.3±1.3
HR (bpm)	-14.9±3.7	-20.9±9.2
FBF (ml/min)	3.0±1.4	0.7±0.4

P2-017

The Role of Estrogen in Effects on Blood Pressure and Vascular Function Induced by Maternal Protein Restriction in Rat Offspring Yuka Musha, Shigeru Itoh, Katsuyuki Kinoshita; Department of Obstetrics and Gynecology, School of Medicine, Juntendo University, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421 Japan

It is established that there are gender-related differences in the effects on offspring blood pressure induced by maternal protein restriction in animal studies. Since such effects may depend on estrogen levels, we hypothesized that lower estrogen would induce an earlier onset of hypertension caused by maternal undernutrition. Wistar rats were fed a diet containing either 18% (C) or 9% (R) casein throughout pregnancy. Half

of the offspring in both C and R groups were ovariectomized on day 50 (CX, RX), and the other half underwent a sham operation (CO, RO). On d175, offspring were killed for small artery reactivity and histological investigation. Birth weight and later growth were not significantly different between C and R. RX had higher systolic blood pressure than CX on d125, but no difference was seen between RO and CO. On d175, systolic blood pressure was higher in R than in C, whether or not ovariectomized. Dilator responses to acetylcholine and bradykinin in small mesenteric arteries were significantly attenuated in RX, although responses to SNP and isoprenaline showed no attenuation in R. The ratio of coronary perivascular fibrosis to total vascular area was higher in R, and the fibrosis became prominent in ovariectomized rats. These findings suggest that estrogen plays an important role in limiting the elevation of offspring blood pressure induced by maternal undernutrition, possibly via BK–mediated mechanisms. The processes may underlie gender and lifecourse patterns of hypertension and also the developmental origins of this disease.

P2-018

Social Class Gradient Related to Blood Pressure Among Indian Adolescents

Shobha Rao, Asawari N. Kanade and Priti P. Apte; Biometry and Nutrition Group, Agharkar Research Institute, G.G. Agarkar Road, Pune 411 004, India.

Background: There is a growing concern that the association between low birth weight and development of hypertension may be confounded with postnatal growth influences. Limited human studies have examined the issue with respect to rapid gain, accelerated growth or childhood obesity; while more relevant worry in developing countries is growth faltering that often results in stunting. We examined association of stunting with blood pressure in Indian adolescents from low and high socio-economic (LSE, HSE) classes. **Methods**: Adolescent boys (9-17 yr) from schools catering to LSE (996) HSE (1146) populations were measured for anthropometry (weight, height, body mass index, circumferences and skinfolds) and blood pressure (systolic- SBP and diastolic -DBP) using standard measures. **Results**: Boys from LSE were significantly ($p<.001$) shorter and thinner compared to those from HSE at all age groups and prevalence of overweight (by International Obesity Task Force criterion) was significantly ($p<0.000$) lower (1.8% Vs 24.7%). However, prevalence of high DBP was significantly ($p<0.05$) higher among LSE (10.5%) while that of high SBP was higher (12.0) among HSE children. As the prevalence of stunting (Z score < -2) was higher in LSE (27.3%) than in HSE (2.9 %) children, we further examined its association with DBP. Prevalence of high DBP remained higher than prevalence of high SBP in stunted children and the difference decreased with improvement in stunting. Prevalence of high DBP remained high (11.4%) even when stunted children were thin (i.e. in the lower tertile of their current weight) while it was not true for prevalence of SBP (1.6%). Additionally, the fact that prevalence of high DBP increased significantly with increase in current weight for children with lower sitting height (<median) indicated that increased vascular resistance may have early origins. **Conclusions**: In view of the fact that association of blood pressure and stroke is stronger in Asians than Westerners and that stunting is a major nutritional problem, our findings underscore need for public health initiatives to reduce stunting in early life.

P2-019

Blood Pressure During Physiological Stress in Adults After Prenatal Exposure to the Dutch Famine R.C. Painter*, S.R. de Rooij*, T.J. Roseboom*, P.M.M. Bossuyt*, D.I.W. Phillips#, C. Osmond#, D.J.P. Barker§, O.P. Bleker##. * Department of Clinical Epidemiology and Biostatistics, Academic Medical Center at the University of Amsterdam, Amsterdam, the Netherlands # MRC Epidemiology Resource Centre at the University of Southampton, Southampton, UK § Developmental Origins of Adult Disease Centre, University of Southampton # # Department of Obstetrics and Gynecology, Academic Medical Center at the University of Amsterdam, Amsterdam, the Netherlands

Background Prenatal exposure to maternal famine in early gestation is associated with a threefold increase in coronary heart disease at age 50. Programming of stress response may explain the link between famine exposure an subsequent coronary heart disease. **Methods** We performed continuous blood pressure and heart rate measurements during a battery of three 5-minute physiological stress tests (Stroop test, mirror-drawing test and a public speech task) in 723 men and women, aged 58, born as term singletons in Amsterdam around the time of the Dutch 1944-1945 famine. **Results** During the stress tests, the systolic blood pressure rose from baseline by 18 mmHg during the Stroop test, by 30 mmHg during the mirror-drawing test and by 46 mmHg during the public speech task. Men were more stress responsive than women (2.0 mmHg extra systolic increase, $p=0.03$). Systolic and diastolic blood pressure increase during stress were highest among people exposed to famine in early gestation compared to unexposed subjects (3.2 mmHg extra systolic increase adjusted for sex, $p=0.06$; 1.3 mmHg diastolic increase adjusted for sex, $p=0.1$). Exposure during mid and late gestation was not associated with stress-related increment of blood pressure (p adjusted for sex >0.7). Correcting for potential confounders (baseline blood pressure, anti-hypertensive medication use, smoking, adult body mass index and size at birth) in a multivariable model did not attenuate the association between famine exposure in early gestation and systolic and diastolic blood pressure increment. Heart rate increment was not related to famine exposure during any part of gestation. **Conclusion** We found a more pronounced blood pressure increase during stress among people exposed to famine in early gestation. Increased stress responsiveness may underlie the known association between coronary heart disease and exposure to famine in early gestation. In future studies we aim to determine the mechanisms responsible for stress response after prenatal exposure to the Dutch famine.

P2-020

Neonatal Dietary Intake and Autonomic Responsiveness to Orthostatic Stress at 7-8 Years Rakesh Sahni[a], Kiyoko Ohira-Kist[a], Sudha Kashyap[a], Micheal M Myers[ab], and Karl F Schulze[a]; Departments of Pediatrics[a] and Developmental Psychobiology[b], College of Physicians and Surgeons of Columbia University, New York, NY 10032 USA

Background: In animals, dietary modifications during early development induce both short- and long-term changes in blood pressure (BP). However, in preterm infants prospective dietary studies of different protein and energy intakes and rates of postnatal growth have failed to demonstrate any differences in BP at 7.5-8 years of age. Whether postnatal early nutritional experiences in preterm infants alter autonomic responsiveness later in life is not known. **Objective**: To evaluate the effects of neonatal dietary intake on BP and cardiovascular orthostatic stress responses at 7-8 years. **Methods:** *Twenty-two low birth weight (birth weight 750-1600 g) infants were studied at 7 to 8 years of age. The infants were fed one of five rigidly* controlled enteral diets from first enteral feeds until discharge. The diets delivered similar amounts of protein but differed in the absolute amounts and relative proportions of fat and carbohydrate (CHO). Following discharge from the neonatal unit dietary intake was not regulated. BP and heart rate (HR) measurements were made at rest and following one and two minutes of orthostatic stress (supine to standing), using an Accutorr oscillometric BP device and an appropriately sized cuff. The children were divided into two groups based on neonatal fat and CHO intakes: Group A (fat<6.5 and CHO>10.7 g/k.d) and Group B. (fat>6.5 and CHO<10.7 g/k.d). Changes in BP's and HR following 1 and 2 min of orthostatic stress were then compared between the two groups using ANOVA. **Results:** The two groups A (n=9) and B (n=13) differed in neonatal fat (5.3±0.7 vs. 8.3±0.8 g/k.d, $p<0.00001$) and CHO (17.0±3.2 vs. 9.8±1.0 g/k.d, $p<0.00001$) intakes respectively. Birth weight, gestational age and total energy intakes were similar. At 7-8 years, no differences in weight, height, body mass index, resting (sitting) BP's and HR were observed between the two groups. However, children given high fat/low CHO diet (Group B) demonstrated greater decreases in systolic (-13.6±2.0 vs. -5.2 ±6.1 mmHg, $p<0.001$), diastolic (-5.8±6.0 vs. -1.3±7.7 mmHg, $p=0.17$) and mean (-12.4±7.1 vs. -3.3±6.1 mmHg, $p<0.005$) BP's at 1-min following orthostatic stress as compared to Group A. The HR increases with standing tended to be greater in Group B at one (6.4±6.2 vs. 3.6±4.7 bpm, $p=0.2$) and two (8.4±6.8 vs. 4.2±3.2 bpm, $p=0.06$) min respectively. On multivariate post-hoc analyses, these group differences were predominantly due to different fat intake during the neonatal period. **Conclusions:** Our data demonstrate that higher dietary fat intake during the neonatal period is associated with diminished BP compensation to orthostatic challenge at 7-8 years of age. These differences suggest that early postnatal nutritional experiences can alter autonomic responsiveness at 7-8 years and provide evidence that may link early postnatal nutritional practices in low birth weight infants to vulnerability to adult cardiovascular diseases.

P2-021

Child Blood Pressure and Change in Weight for Age Z Score from Birth to Childhood: Aboriginal Birth Cohort 1987-2001 Susan Sayers, Dorothy Mackerras, Gurmeet Singh Menzies School of Health Research, Institute of Advanced Studies, Charles Darwin University Darwin, Northern Territory, Australia

Introduction: The inverse relationship of birth weight with later blood pressure (BP) is well reported with the most frequent finding being that risk is greater in those who were relatively smaller at birth (small for gestational age) but larger as adults. These results are interpreted as an effect of post natal growth **Aim :** To study the relationship of child blood pressure with change in weight for age z score from birth to childhood. **Methods: Design:** A longitudinal prospective birth cohort study **Setting:** Top End of the Northern Territory of Australia **Subjects:** 449 Aboriginal children who were born at term and examined at a mean age of 11.4 years; 50.5% were boys and 50.5% had commenced puberty. Small (SGA) and appropriately grown for gestational age (AGA) were defined as < 10^{th} percentile and = 10^{th} percentile respectively of birth weight for sex using the 1978 NCHS growth reference. There were 63 SGA babies and 386 AGA babies. **Outcome measures:** Association between BP and change in weight for age z score (WAZ) between birth and 11 years of age. **Results:** At birth, 63 (14%) of term children were SGA whereas 10% had WAZ>90^{th} percentile. At 11 years of age, 44 % of children had experienced decline in WAZ >0.66 since birth whereas 22% had experienced an increase of >0.66. Change in WAZ was inversely related to birth WAZ ($r=0.48$, $p<0.001$). Overall SGA babies had a lower BP than AGA babies ($p=0.001$). BP was correlated with change in z score ($p<0.001$) and these effects were independent. This result did not change if 45 children with birth weight >90^{th} percentile were excluded. Systolic blood pressure at 11 years by birth weight category and change in WAZ from birth to 11 years: Aboriginal Birth Cohort 1987-2001

WAZ difference	AGA		SGA	
	N (%)	Mean systolic BP, mmHg	N %	Mean systolic BP, mmHg
Loss > - 0.66	195 (50%)	104.4	3 (5%)	101.7
Between -0.66 and 0.66	126 (33%)	108.4	25 (37%)	103.6
Gain > 0.66	65 (7%)	115.8	35 (56%)	110.7

Conclusions: Overall the participants had poor postnatal growth with a negative change of WAZ from birth to 11 years of age. Contrary to the prevailing hypothesis the highest blood pressures did not occur in the small term babies who had substantial gain

in relative weight. In view of high prevalence of relative weight loss further follow-up of this cohort will be of interest.

P2-022

The (Un)Stressed Gene Hypothesis Allan D. Sniderman, Mike Rosenbloom Laboratory for Cardiovascular Research, McGill University, Montreal, Quebec, H3A 1A1, Canada

The risk of cardiovascular disease is rising steeply amongst many of the peoples of South East Asia. The Thrifty Gene Hypothesis has been proposed as an explanation for these major changes in disease frequency. We suggest an alternative explanation: The (Un)Stressed Gene Hypothesis. There is broad agreement that the two external drives for these changes in disease patterns are first, an increase in energy intake due to major dietary changes and second, a decrease in energy output due to changes in work requirements. The consequence of these external changes is energy excess which is expressed internally by dyslipidemia and diabetes. The dyslipidemia is characterized not by hypercholesterolemia but by hypertriglyceridemia and increased numbers of small dense LDL- that is, by hyperTg hyperapoB. Broadly speaking, adipose tissue can be separated into two zones: lower body (LB) and upper body (UB). LB adipocytes are the primary zone of adipose tissue development during maturation. UB adipocytes are the primary zone of postmaturation adipose tissue expansion. The metabolic complications of obesity- particularly hyperTg hyperapoB- are more common with expansion of the UB adipose tissue mass. The (Un)Stressed Gene hypothesis states that many of the peoples of South East Asia have reduced numbers of LB adipocytes compared to many Caucasians. Consequently, they have a reduced capacity to store excess energy in the LB and that leads to earlier expansion of UB adipose tissue mass with all the adverse consequences that follow. Differences in the external temperature may have been the driving evolutionary force leading to these differences in LB adipocyte number with Caucasians needing greater capacity to retain heat and peoples of South East Asia needing greater capacity to lose heat.

P2-023

Intrauterine Growth Restriction and Risk of Hypertension: a Fifty Year Follow Up Dale Spence[1], Fiona A. Alderdice[1], Moira C. Stewart[2] and Henry L. Halliday[2]; School of Nursing & Midwifery, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL[1], Department of Child Health, Queen's University Belfast, Grosvenor Road, Belfast BT12 6BJ[2]

Background: Intrauterine growth restriction (IUGR) remains a major clinical problem in obstetric practice. Advances in neonatal intensive care have resulted in an increased survival rate of a heterogeneous group of babies, including those with IUGR. IUGR however, is still associated with a significantly higher perinatal mortality. In addition, it is now widely accepted that the risks of various chronic diseases in adulthood including hypertension, may have their origins before birth. **Methods**: Birth records of babies born in the 1950s in Royal Maternity Hospital, Belfast were accessed and used to identify an IUGR study group (n = 108) and a comparison group (n = 124). The study objectives were to compare health related quality of life, general health, health service use, socio-economic status, blood pressure and anthropometric measures in 50 year old adults who were born at term (= 37 weeks' gestation) with IUGR (< 10^{th} centile) and a group born at term not growth restricted (> 10^{th} centile), using a retrospective cohort design. The data collected included physical, biological, psychological and socio-economic aspects over the lifecourse, using validated questionnaires and individual physical assessment. This presentation will focus on the findings regarding blood pressure. The participant sat in a comfortable position and the European recommendations for measuring blood pressure were followed using an automated monitor with a choice of cuff size. The average of two readings was used, with a five-minute interval before the first reading and a two-minute interval between the first and second recordings. Adjustments were made for potential confounding variables including gender, social class at birth, marital status, education and Townsend Deprivation Index. These reflected a range of social variables throughout the individual's lifecourse, which may have introduced chance confounding. **Results:** Participants in the study group had significantly higher systolic blood pressure (SBP) and diastolic blood pressure (DBP) than those in the comparison group (Table). In addition, more participants in the IUGR group were receiving treatment for high blood pressure. Regression analysis of potential confounding variables and explanatory variables (body mass index, smoking and alcohol intake) did not explain the difference between groups.

	Study Group (n = 108)	Comparison Group (n = 124)	P Value (2-tailed)
SBP (mmHg)*	131 (19)	127 (16)	0.05
DBP (mmHg)*	82 (14)	79 (11)	0.05
*Mean (SD)			
Have high blood pressure (Yes/No)		Odds Ratio (95% CI)	P Value
Unadjusted		1.84 (1.06 to 3.18)	0.03
Adjusted for all confounders		1.98 (1.11 to 3.54)	0.02

Conclusions: IUGR is associated with higher blood pressure at age 50 years. Resources should be targeted at effective and comprehensive blood pressure screening programmes beginning in early adult life for those who were born with IUGR. Future research should continue to focus on IUGR using a lifecourse and intergenerational approach, to shed light on mechanisms at work in the fetal origins of adult disease. Further follow up of this cohort would determine if there is amplification with time of disease in those born with IUGR.

P2-024

Maternal Blood Pressure and Fetal Heart Rate in Preeclamptic and Normotensive Pregnancies* M.L. Swansburg, C.A. Brown, G.N. Smith, & B.S. Kisilevsky; Department of Obstetrics & Gynecology, School of Nursing, Queen's University, Kingston General Hospital, Kingston, ON, Canada

Background: Preeclampsia (PE) during pregnancy contributes to adult risk for cardiovascular disease through two paths. It is associated with: 1) long term maternal risk of developing cardiovascular disease and 2) fetal adaptation (e.g., growth restriction) in these pregnancies that may lead to the development of cardiovascular risk factors (e.g., increased cholesterol, hypertension) in the offspring. Although the maternal condition influences fetal outcome, to date, few studies have examined the effects of maternal cardiac function on fetal cardiac function, the focus of this study. A better understanding of the cardiovascular changes occurring in the woman with PE and its effect on the cardiovascular system of the fetus is needed to identify those factors in-utero which may contribute to 'programming' for adult disease. **Methods**: Thirty-three mother-fetal pairs (n=22 normotensive (NORM), n=11 PE) from 32 to 40 weeks gestational age (GA) were recruited for fetal testing on one occasion as part of a larger, longitudinal study of PE. The highest maternal antenatal blood pressure (BP in mmHG) at 25 (± 3), 34 (± 3) and 39 (± 1) weeks (±SD) gestational age (GA) as well as maternal body mass index (BMI), prepregnancy and before delivery, were obtained from the antenatal records. At 36 weeks (± 2 weeks) GA, each fetus had a 20 min continuous recording of spontaneous heart rate (FHR) using a cardiotocograph. **Results**: Women with PE had a higher (p<.01) pre-pregnancy BMI (M=29.7 vs. M=23.5, PE vs. NORM) and delivery BMI (M=36.2 vs. M=29.3, PE vs. NORM). Subsequent analyses controlled for BMIs. Mixed model ANOVAs to determine differences in BP over GA (25, 34, & 39 weeks) between Groups (PE vs. NORM) showed that systolic BP increased over gestation in both groups (M = 123, 141, 147 vs. M = 122, 125, 128) with PE women demonstrating a greater increase (p<.03). Diastolic BP increased over gestation (p<.03, M=76, 84, 96 vs. M=72, 75, 80) and the PE group had a higher overall average (p<.004). Fetuses in PE vs. NORM pregnancies exhibited fewer (p<.01) FHR accelerations ≥ 10 bpm (M=3.6 vs. M=7.6) and ≥15 bpm (M = 1.0 vs. M = 3.3) and fewer FHR decelerations ≥10 bpm (M =0.6 vs. M= 2.1). Stepwise regression with FHR accelerations ≥ 10 bpm as the dependent variable and maternal BPs and BMIs as the independent variables showed negative relations (p<.05) between number of FHR accelerations ≥ 10 bpm with 39 week GA maternal systolic (r = -.40) and diastolic BP (r = -.35); the 39 week GA systolic BP was retained in the model as a significant predictor of number of FHR accelerations (r^2 = .16). Regression analysis with FHR accelerations ≥ 15 bpm showed negative relations (p<.05) with maternal delivery BMI (r = -.38) and 39 week GA systolic (r = -.44) and diastolic BP (r = .40); again only 39 week GA systolic BP was retained in the model (r^2 = .20). **Conclusions**: These preliminary findings suggest a relation between maternal and fetal cardiac function with the number of FHR accelerations decreasing as maternal BP increases following diagnosis of PE. It was concluded that atypical fetal cardiac function in pregnancies complicated by preeclampsia may be an early marker of adaptive autonomic nervous system development which parallels or reflects fetal programming. *Funded by a grant from the Canadian Institutes for Health Research and the Canadian Heart & Stroke Foundation to G.N. Smith & B.S. Kisilevsky. M.L. Swansburg was supported by an Ontario Graduate Student Award.

P2-025

Early Alteration of Arterial Stiffness in Premature Infants L. Tauzin*, P. Rossi°, B. Giusano**, A. Boussuges °, A. Fraisse***, U. Simeoni*. Department of Neonatology(*), Department of Medical Information(**), Pediatric Cardiology Unit(***), Division of Internal Medicine and Emergency(°), Assistance Publique, Hôpitaux de Marseille and Faculté de médecine, Université de la Méditerranée, Marseille, France.

Background: Premature birth is a factor of raised blood pressure in adulthood. Little is known about the characteristics of arterial bed in neonates. **Aim:** To characterize in vivo the arterial compliance in neonates and its maturation profile in premature infants. **Patients and Methods:** Eleven stable premature infants (gestational age: 28.6 +/- 0.6 weeks) were compared to eleven babies born near term (gestational age: 35.9 +/- 0.8 weeks). Abdominal aortic wall distensibility coefficient and whole body arterial compliance were calculated using conventional ultrasonic measurements including the abdominal aorta cross area section, in systole and diastole, and the left ventricle stroke volume, in combination with simultaneous non-invasive recording of aortic pulse pressure. Measurements were performed on the 5th day of life and were repeated on the 7th week of life in premature infants. **Results:** At birth, whole body arterial compliance and distensibility coefficient in premature infants were lower than in controls (4.9 +/- 1.3 vs 7.8 +/- 2 $mm^3/kPa/m^2$, p<0.002 and 55.1 +/- 11.6 vs. 111.5 +/- 23.3 10^{-6}/kPa, p<0.001 respectively). Furthermore, in premature infants, whole body arterial compliance and distensibility coefficient remained unchanged on the 7th week after birth (respectively 5.3 +/- 1.5 $mm^3/kPa/m^2$, p=0.3 and 65.3 +/- 21.5 10^{-6}/kPa, p=0.2). Last, whole body arterial compliance and distensibility coefficient in premature infants at 7 weeks were lower than in controls (p<0.005). **Conclusions.** Premature infants are characterized as early as the 5th day of life by a high arterial stiffness, that persists when they reach their theoretical term.

P2-026

Reproductive history and cardiovascular mortality among women. Evidence from the Uppsala Birth Cohort Multigeneration Study (UBCoS Multigen) Denny Vågerö, Bitte Modin, Ilona Koupil; Centre for Health Equity Studies (CHESS), Stockholm University/Karolinska Institute, 106 91-Stockholm, Sweden;

Background: Circulatory disease may be more common in women with a history of many child births. Previous studies have suggested that their all-cause as well as circulatory mortality is raised. An important question is whether or not repeated childbirths in themselves impose a future risk on women, or if any excess risks are due to the social circumstances of women and families with many children. We address that question by analysing and comparing female and male mortality experience by the number of children they had. In further analyses we also took into account information about their foetal growth. **Methods**: We used data on all women and men born in Uppsala Academic Hospital 1915-1929 (the Uppsala Birth Cohort: UBCoS) combined with information on any children born to them. The study is based on those 12,168 UBCoS women and men who were resident in Sweden in 1947, and their completed childbirths, traced through the Swedish Multigeneration Registry. Information on the cohort members' birth characteristics was abstracted from obstetric records. Mortality was followed up until 2001 through linking to the Swedish Cause-of-Death Registry. **Results**: Women and men with 5+ children had a somewhat higher all cause mortality than women and men with 1-4 children. Relative risks were 1.27 (95% conf.limits 0.96-1.68) for women and 1.20(0.92-1.57) for men. Looking specifically at circulatory mortality we found higher mortality from ischaemic heart disease (IHD), but not from cerebrovascular disease, among women with 5+ childbirths than among women with 1-4 childbirths. For women with many births, relative risks of IHD were 2.03 (1.28-3.22). In contrast, men with many children had no significant excess risk from IHD , with RR=1.25 (0.88-1.77). Relative risks of cerebrovascular death were elevated neither for women (RR=0.97) nor for men (RR=0.68) with many children. Adjusting for birth weight for gestational age did not change these results. **Conclusions**: Carrying and giving birth to many children may impose an excess risk for ischaemic heart disease on women. In contrast, no such risk could be shown for men fathering 5+ children. We discuss whether this a true gender difference, or due to differential selection into parenthood. A further research question is whether IHD mortality risk accumulates with each pregnancy or if it is only present after a very high number of pregnancies.

P2-027

Relationships of Maternal and Paternal Birthsize to Cardiovascular Disease Risk in the Adult Offspring: An Intergenerational Study in South India SR Veena, S Geetha, K Kumaran, J Saperia, DJ Fisher, P Coakley, CE Stein and CHD Fall; Research Centre, Holdsworth Memorial Hospital, Mysore-570021, South India, Dorset and Somerset Health Protection Unit, Wellsprings Road, Taunton, TA27PQ, UK, MRC Epidemiology Resource Centre, Southampton General Hospital, Southampton, SO16 6YD UK.

Background Several studies in diverse population have found association between low birthweight and an increased risk of coronary heart disease (CHD) and type2 diabetes in adult life. These associations may reflect programming effects of fetal undernutrition ('fetal origins' hypothesis) and/or common genes causing low birthweight and adult disease ('fetal insulin' hypothesis). We have examined this issue by studying the relationship between maternal and paternal birthweight and CHD risk factors in the adult offspring in South India. **Methods** We identified 415 mother offspring pairs and 296 father offspring pairs where both the parent and child were born in Holdsworth Memorial Hospital (HMH), Mysore, India, which has preserved birth records since 1934. Investigations in the parents (aged 33-65 years) and offspring (aged 20-46 years) included anthropometry, oral glucose tolerance test, plasma insulin and serum lipid concentrations, blood pressure, electrocardiograph and Rose chest pain questionnaire. Insulin resistance was calculated using homeostasis model assessment. Metabolic syndrome was defined based on WHO criteria. **Results** Among the offspring, lower birthweight was associated with higher120-minute glucose, fasting cholesterol and triglyceride concentrations, higher waist/hip and subscapular/triceps ratios, increased insulin resistance and higher combined prevalence of impaired glucose tolerance, impaired fasting glycaemia and diabetes mellitus (p=<0.01 for all adjusted for sex, age and body mass index). Both maternal and paternal birthweight were inversely related to offspring metabolic syndrome (OR 0.4 (95 % CI: 0.2, 1.0); p=0.057 for mother-offspring pairs; OR 0.3 (95 % CI: 0.08, 0.9); p=0.031 for father-offspring pairs). Paternal but not maternal birthweight was inversely related to offspring insulin resistance (e^{β}= 0.9 (95 % CI: 0.8, 1.08; p=0.3 for mother-offspring pairs; e^{β} = 0.8 (95 % CI: 0.7, 1.01; p=0.06 for father-offspring pairs). We also found inverse relationships between maternal birthweight and offspring systolic blood pressure (ß= -2.5 (95% CI: -5.03, -0.07;p=0.04) and WHR (p=0.03). **Conclusions** In conclusion our study provides support to the fetal insulin hypothesis and indicates that at least part of the association between low birthweight and insulin resistance is genetic in origin. The study also provides evidence for intergenerational effects on vascular disease transmitted through the mother, which are likely to have an environmental component

P2-028

The Effect of Third Trimester Fetal Growth on Blood Pressure and Lipid Profile in Adolescence Signe E.Vielwerth[1], Rikke Beck Jensen[2], Torben Larsen[3], Allan Vaag[4] and Gorm Greisen[1]; 1: University Department of Neonatology, Rigshospitalet, Denmark, 2: University Department of Growth and Reproduction, Rigshospitalet, Denmark, 3: Department of Gynaecology and Obstetrics, Holbaek Sygehus, Sygehus Vestsjaelland, Denmark, 4: Steno Diabetes Center, Gentofte, Denmark.

Background: Birth weight is a surrogate measure of fetal growth; to evaluate fetal growth pattern, longitudinal measurements are required. In studies assessing the hypothesis of "Fetal Origins of Adult Disease", birth weight is the most commonly available information to elucidate the relation between the intrauterine environment and health later in life. Intrauterine growth restriction (IUGR) is assumed to be the cause of the increased risk of adult disease in individuals with a low birth weight for gestational age (SGA). The present study holds information on longitudinally recorded fetal growth velocity in the third trimester, the period where placental function becomes critical for fetal growth. **Aim:** The aim of this study was to investigate the effect of third trimester growth upon blood pressure and lipid profile in adolescence. **Methods:** One thousand pregnant women were enrolled in a previous study on the basis of an elevated risk of giving birth to a child small for gestational age (SGA). Intrauterine growth velocity in the third trimester was calculated from longitudinal ultrasound measurements including one dating scan and estimated fetal weight from gestational week 27 until birth. Intrauterine growth restriction (IUGR) was defined as relative intrauterine growth velocity in third trimester below the 10% centile. Intrauterine growth velocity could be calculated in 594 cases and of these, 267 were invited for follow up at adolescence. Blood pressure, cholesterol and triglyceride levels were measured in offspring at mean age 17.5 years (range: 16.2 – 19.4 years). Of the 123 subjects who agreed to participate, 44 were IUGR and 79 were not IUGR. The study received ethical approval and informed consent was obtained. **Results:** There was no significant difference in systolic blood pressure, diastolic blood pressure, cholesterol or triglyceride levels between the group who had been growth restricted (+IUGR) in the third trimester and the group who had not been restricted (-IUGR). **Discussion:** The two groups (+/– IUGR) had a similar mean birth weight deviation, and the lack of a significant effect of IUGR on the outcome parameters suggests that intrauterine growth velocity in third trimester does not contribute to the association between birth weight and later health. However the individuals examined in this study are young, and it is possible that an effect of IUGR will be evident at a later age or in a larger cohort. **Conclusion:** This study does not suggest an effect of IUGR upon cardiovascular risk factors (blood pressure and lipid profile) in adolescence.

Table 1	-IUGR (mean +/- SD)	+IUGR (mean +/- SD)	P
Birth Weight SDS	-0.59 +/- 1.19	-0.70 +/- 1.25	0.64
Mean systolic blood pressure	122.2 +/- 11.1	120.4 +/- 10.2	0.37
Mean diastolic blood pressure	70.08 +/- 8.20	71.05 +/- 7.33	0.52
Cholesterol (mmol/l)	3.94 +/- 0.92	4.11 +/- 0.82	0.31
HDL (mmol/l)	1.40 +/- 0.33	1.41 +/- 0.33	0.94
LDL (mmol/l)	2.55 +/- 0.78	2.44 +/- 0.69	0.53
LDL/HDL ratio	1.77 +/- 0.71	1.82 +/- 0.66	0.71
Triglyceride (mmol/l)	0.91 +/- 0.47	1.03 +/- 0.50	0.20

P2-029

Decreased Sympathetic Tone in Fetal Growth-Restricted Male Adult Mice Carole S Watson, Alan D Bocking, Victor KM Han, and S Lee Adamson. Ob/Gyn and Physiology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Toronto and Paediatrics, Child Health Research Institute, University of Western Ontario, London, Canada.

Rationale: The sympathetic nervous system is important in the regulation of cardiovascular activity and is susceptible to developmental perturbations during fetal life which may lead to permanent alterations in adulthood. Adults with low birth weight have been shown to have lower sympathetic nerve activity, but reports are conflicting. The insulin-like growth factors (IGF-I and –II) are important in the development of the nervous system, regulating both proliferation and apoptosis, and their effects are inhibited by the IGF binding proteins (IGFBP). We have developed transgenic (Tg) mice which overexpress IGFBP-1 and are growth-restricted at birth. The purpose of this study was to analyze sympathetic regulation of cardiovascular activity in these Tg mice. **Methods:** HR was measured following β-adrenergic blockade to assess sympathetic tone (propranolol 6 mg/kg ip) or parasympathetic blockade to assess vagal tone (atropine 4 mg/kg ip). Arterial blood pressure (BP) and heart rate (HR) were measured by tail-cuff plethysmography. **Results:** Female Tg mice were hypertensive at 5 weeks of age compared to WT, becoming relatively hypotensive by 12 weeks. Male Tg mice were hypertensive at 8 weeks. The BP of male and female Tg mice was not different from WT at 6 months to 1 year of age. Resting HR in male and female Tg mice was significantly lower than WT by 5-17% at all ages studied (Table below). Following propranolol administration, HR decreased by a lesser percent in Tg male mice as compared to WT males (Tg 78±2 vs WT 71±2% of baseline (P<0.01), n=10). However, in female mice, HR decreased by a similar amount (Tg 74±3 vs WT 76±2% of baseline, n=6). There was no change in HR in either group after atropine administration ** P<0.01, * P<0.05; Tg compared to WT §§ P<0.01, § P<0.05; compared to 5 weeks.

	Male				Female			
	Arterial Pressure (mmHg)		Heart Rate (bpm)		Arterial Pressure (mmHg)		Heart Rate (bpm)	
Mean ± SEM	Tg (n=18)	WT (n=13)	Tg (n=18)	WT (n=13)	Tg (n=20)	WT (n=10)	Tg (n=20)	WT (n=10)
5 Weeks	109±3	110±5	556±11**	666±16	124±6*	113±4	571±13*	636±29
8 Weeks	126±7*‡	112±3	603±16**‡‡	707±22	116±3	117±4	614±11**‡	670±14
12 Weeks	106±3	109±3	637±15**‡‡	722±15‡‡	105±2*‡	111±3	646±11**‡‡	746±17‡‡
24 Weeks	110±3	105±5	676±9**‡‡	704±10	105±3	102±6	671±9**‡	742±11‡‡
36 Weeks	113±2	110±4	665±12**‡‡	700±16	104±2	104±3	679±9**‡‡	737±14‡‡
52 Weeks	116±4	115±3	647±8**‡‡	705±13‡‡	102±3	105±4	676±12**‡‡	724±9‡‡

Conclusions: Despite a normal BP, these mice maintain a lower heart rate. The current study indicates that male mice, which are growth-restricted due to elevated IGFBP-1, have decreased sympathetic tone while females do not. We conclude that this model of IUGR does not result in sustained adult hypertension but does demonstrate permanent alterations in HR control possibly due to alterations in the development of the sympathetic nervous system in male mice.

P2-030

Birth Weight As Risk Factors For Stroke Among Working Age Women

Elisabete Weiderpass[1,2], Ming Lu [2,3], Weimin Ye[2], Hans-Olov Adami[2]
1. The National Cancer Registry of Norway, Oslo, Norway 2. Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden, 3. Unit of Clinical Epidemiology, Qilu Hospital of Shandong University, Jinan, P.R. China

Background: Previous studies on the relation between birth weight and the risk of stroke show inconsistent results. **Method:** In 1991 and 1992, Women's Lifestyle and Health (WLH) Cohort Study was initiated in Sweden. Through linkage with several nationwide registries, 45,450 women, who were free of stroke at entry, were followed until diagnosis of first incident stroke, death, emigration out of Sweden, or the end of follow-up (December 31, 2002), whichever occurred first. Hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs) were derived from Cox proportional hazards regression model. **Results:** We confirmed 202 incident cases, of which 140 were ischemic stroke (IS), 48 intra-cerebral haemorrhages (ICH), and 14 undefined. The participants reported their birth weight at enrolment. Compared to the 'normal baby' with a birth weight between 2.5 and 3 kilogram, the 'small baby' with a birth weight less than 2.5kg and the 'big baby' whose birth weight over 3000g tended to have no effect on the risk of stroke, neither ischemic stroke nor hemorrhagic stroke. The age adjusted HR for all stroke was 0.8 (CI 0.4-1.6) for birth weight below 2.5 kg and 0.9 (CI 0.7-1-2) for birth weight over 3 kg, compared with birth weight of 2.5-3.0 kg. For ischemic stroke the age adjusted HR were 0.8 (CI 0.3-1.8) for birth weight less than 2.5 kg and 0.9 (CI 0.7-1.5) for birth weight over 3 kg compared to birth weight 2.5-3 kg. For haemorrhagic stroke the age adjusted HR were 0.6 (CI 0.1-2.4) for birth weight less than 2.5 kg and 0.6 (0.3-1.0) for birth weigh over 3 kg, compared to birth weight 2.5-3.0 kg. Further adjustments in the model (for smoking, alcohol intake, age at menarche, education, physical activity, history of hypertension and diabetes mellitus) did not change these results substantially. **Conclusions:** In this large prospective study among women below age 60 at end of follow-up, we found no clear association between birth weight and risk of ischemic or haemorrhagic stroke.

P2-031

Glucocorticoids and Programming—Evidence for Different Effects Depending on the Steroid Used

E.Marelyn Wintour*, Miodrag Dodic,* Robert de Matteo*, A.McAlinden* Andrew Jefferies*, Debbie Bartal*, Karen Moritz**.*Dept.of Physiology, and ** Dept of Anatomy and Cell Biology, Monash University, Clayton, Victoria, 3800, Australia.

It has been reported that there are differential direct effects of maternally – administered cortisol and betamethasone on fetal lung maturation in sheep[1]. There are also differential effects of betamethasone and dexamethasone, administered during the last week of pregnancy, to maternal rats, on the long-term blood pressure of the offspring [2,3]. In the current report we discuss the differential effects of early (26-28day, term = 145-150d) treatment of pregnant ewes with dexamethasone (0.48mg/h) or cortisol (5mg/h) on the long-term physiology of the male and female offspring. *Mechanism of hypertension* In both sexes the adult offspring of both treatments develop higher blood pressure than control, saline-treated animals[4,5] at 1-2years of age. There is evidence that this is due primarily to increased cardiac output in adult females treated in utero with dexamethasone[6]; recent results suggest that this is not so for adult males treated in utero with cortisol. In such adult male offspring there was a tendency for cardiac output (CO) to be decreased, and total peripheral resistance (TPR) to be increased. This was accompanied by a statistically significant decrease in mean arterial pressure (MAP), with propranolol and phentolamine blockade, and a significant decrease in both MAP and TPR with hexamethonium blockade. Thus there was evidence that the increased sympathetic control of peripheral vasculature in the cortisol-programmed adult males. *Brain* In adult dexamethasone-programmed males there was an increased pressor responsiveness to angiotensin II (1,10ug/h) infused into the lateral ventricle, compared to the saline-treated offspring, but this was not seen in the adult male offspring of early cortisol-treatment. This correlated with changes in gene expression in the brain renin-angiotensin system of late term fetuses, of both sexes, of the two different steroid treatments—there being up-regulation of hypothalamic angiotensinogen and medullary AT1 expression in dexamethasone-programmed, but not cortisol programmed offspring [4,5] *Kidney* Both steroid treatments resulted in qualitatively similar decreases in nephron endowment, and time-related changes in expression of some genes (RAS). However, in the adult female offspring there were subtle differences in the renal handling of salt.
Conclusions Synthetic and natural glucocorticoids have differing affects on mineralo- and glucocorticoid receptors (MR,GR) and on PXR. These differences are particularly evident in the brain, and may underlie the differential effects of dexamethasone and cortisol as programming steroids.

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P2-032

Transient Neonatal Hyperoxia Induces Hypertension, Vascular Dysfunction and Microvessel Rarefaction in Adult Rats

Catherine Yzydorczyk, Gilles Cambonie, Patrick Pladys, Isabelle Lahaie, Daniel Abran, Fernand Jr Gobeil[1], Pierre Hardy and Anne Monique Nuyt Research Center, Hôpital Sainte-Justine, Department of Pediatrics and Pharmacology, Université de Montréal, H3T 1C5, [1]Department of Pharmacology, University of Sherbrooke, Canada.

Background: Recent reports have emphasized the impact of gestational age on blood pressure in adult life, independently of birth weight. Premature babies with chronic lung disease are also at increased risk of hypertension (HT). The mechanisms underlying these observations are currently not understood. In adults with chronic HT, vascular dysfunction is associated with oxidative stress and one of the major mediators contributing to reactive oxygen species generation is angiotensin II. Premature infants have indices of increased oxidative stress and are more susceptible to oxidant injury to their developing vessels (such as retinopathy of prematurity and periventricular leukomalacia). The current studies were undertaken to verify the hypothesis that perinatal oxygen radical injury can lead to long-term cardiovascular consequences with chronic hypertension and vascular dysfunction. **Methods**: Sprague-Dawley rat pups were maintained in a hyperoxic (80% O_2) environment from the third to the tenth day of life. The mother was alternated every day with a surrogate mother that had a litter maintained in normoxia to serve as control (Ctrl). Male were studied and weight and tail cuff blood pressure obtained from 9 to 17 weeks of age. In vivo blood pressure studies in conscious chronically catheterized rats and ex vivo vascular reactivity studies (carotid rings in organ chambers) were realized at 18 weeks of age. **Results**: Pups exposed to O_2 had reduced growth over all the follow up weeks (at 17 weeks: 580 ± 10 vs. 530 ± 4 g Ctrl, n = 13 each group, $p < 0.05$). Blood pressure (measured by tail cuff) was similar at 9 weeks; starting at 10 weeks, diastolic (but not systolic) pressure rose significantly more in rats exposed to O_2 (at 17 weeks: delta of 10 ± 2 mmHg between groups, n = 13 each group, $p < 0.05$). In vivo measured mean arterial blood pressure was significantly increased in the rats exposed to O_2 (115 ± 1 vs. 102 ± 3 mmHg in Ctrl, n = 6 each group). Blood pressure response to angiotensin II infusion was significantly increased and depressor response to sodium nitroprusside infusion significantly decreased in rats exposed to O_2 relative to Ctrl. Ex vivo vasomotor response to angiotensin II was significantly increased in rats exposed to O_2 (Emax relative to maximal response to KCl 80 mM: 168 ± 6 % vs. 129 ± 5 % Ctrl, n = 4 animals each group, $p < 0.05$); no significant difference between the groups were observed in the vasomotor response to cumulative concentration of sodium nitroprusside. Capillary density (at 4 weeks) was significantly decreased in striated muscles (tibialis anterior) from rats exposed to O_2 (918 ± 13 vs. 1319 ± 22 capillaries/mm^2, $p < 0.05$, n = 3 each group). **Conclusions**: Exposure to O_2 in the newborn period leads in the adult to higher blood pressure, vascular dysfunction and microvessel rarefaction at a major site of peripheral resistance i.e. the striated muscle. As microvascular rarefaction preceded elevated blood pressure, we postulate this could represent a primary event in the genesis of hypertension associated with oxidative stress in perinatal life.

Developmental Disruption; Toxic Enviornment

P2-033

Growth Hormone Deficiency in the Neonate Impairs Somatotrope Function and Glucose Metabolism in the Adult Dopaminergic D2R Knockout Mouse

Edith Arany, Isabel Garcia-Tornadu, Sandra M. Thyssen, Jennifer Mc Donald,Graciela Diaz –Torga, David J. Hill and Damasia Becu-Villalobos.

Background: In previous studies we have shown that the dopaminergic D2 receptor male knockout mouse (KO) had a lower GH secretion that their wild type counterpart during the first month of life (Endocrinology, 2002). These mice are growth restricted with lower serum IGF-I. In adulthood, there is no catch up in body weight although serum GH are normal the KO mice. **Objective**: In the present study our aim was to establish if somatotrope function and glucose metabolism were altered in adult KO mice. **Methods**: Pituitary cells were isolated from adult KO mice and cultured in the presence of GHRH (10-7 to 10-9 M) and/or with somatostatin (10-6 to 10-8 M). By immunohistochemistry (ICC) the pituitary gland and the pancreata was analyzed for cell abundance and morphometry. In vivo, GTT was performed to study beta cell response. IGF-I and Pdx-1 expression and abundance was studied by real-time PCR in the pancreas. **Results**: We observed that GHRH induced GH secretion was lower in KO mice ($P < 0.05$), in correlation with a striking decrease in GHRH-induced cAMP generation ($p=0.04$). The effect of somatostatin was not modified by genotype. Using confocal fluorescent immunohistochemistry a decrease in somatotrope population was evidenced in the KO mice ($P=0.0025$). During GTT, glucose levels in the KO were lower than in wild types at 60 and 120 min. Furthermore, insulin levels in response to

GTT, were higher in KO mice at 30, 60 and 120 min. When HOMA values were calculated, at 30 min, units were higher in KO mice indicating impaired insulin sensitivity. Preliminary data on the morphometry of the pancreas (n=3) showed that the adult KO mice had an increased percentage of islet area and increased number of large islets compared with the WT. Furthermore when we analyzed IGF-I, Pdx-1 and glucagon expression in the pancreas by Real-time PCR a dysfunction of the gland was observed, as their levels were lower in the KO mice. These results show that there is a permanent impairment in the somatotrope population and function in D2R KO mice with a concomitant altered glucose metabolism, which may be related by a faulty imprinting of the pancreas due to neonatal GH deficiency.

P2-034

Intrauterine Growth Restriction Late in Gestation in the Fetal Lamb: Effect on the Size of the Macula Densa and the Renal Renin-angiotensin System

Vladislava Zohdi[1], Karen M Moritz[1], Kristen Bubb[2], Megan L Cock[2], Richard Harding[2], M Jane Black[1]. Departments of [1]Anatomy & Cell Biology and [2]Physiology, Monash University, Clayton, Victoria, 3800, Australia

Background: Preliminary observations from our laboratory suggest that the maculae densae in the kidneys of intrauterine growth restricted (IUGR) fetuses are enlarged. Since the maculae densae play a key role in the regulation of renin production, we hypothesised that the renal renin–angiotensin system is up-regulated in the IUGR fetal kidney. **Aims:** to determine (1) whether the volume of maculae densae is increased and (2) whether the renal renin-angiotensin system is up-regulated in the fetal lamb kidney following IUGR in late gestation.**Methods:** IUGR was induced in fetal lambs by umbilico-placental embolization from 110-130 days of gestation (full-term 147days). At 130 days of gestation the ewes and fetuses were humanely killed and the kidneys excised. Samples of cortex and medulla from the left kidney were snap frozen and the right kidney perfusion fixed. In the fixed-kidney, total kidney volume, nephron number, renal corpuscle volume, total maculae densae volume and the volume of macula densa per glomerulus were stereologically estimated. In the frozen kidney, the relative gene expression of the angiotensin II receptors (type 1, AT_1 and type 2, AT_2), renin and angiotensinogen were determined using real time PCR. **Results:** Body weight, kidney volume and nephron endowment were significantly reduced in the IUGR fetuses compared to controls. Interestingly, there was a significant increase in the number of glomeruli per kidney volume in the IUGR group. There was no difference in the total volume of maculae densae between the groups. However, there was a tendency for the volume of macula densa per glomerulus to be higher in the IUGR kidneys. There was a four and five fold increase in AT_1 and AT_2 receptor expression, respectively in the cortex of the IUGR fetal kidneys compared to controls. Angiotensinogen mRNA levels also appeared to be upregulated in the cortex of IUGR fetuses. There was a significant increase in renin mRNA expression in the IUGR renal medulla. **Conclusions:** Induction of IUGR in late gestation leads to reduced birth weight and nephron endowment but up-regulation of the renal renin-angiotensin system.

P2-035

The Effect of Intrauterine Growth Restriction on Coronary Artery Reactivity and Cardiomyocyte Maturation in Fetal Sheep

Kristen J Bubb[1], Megan L Cock[1], Helena C Parkington[1], Miodrag Dodic[1], Richard Harding[1] M Jane Black[2] and Marianne Tare[1]. Departments of [1]Physiology and [2]Anatomy and Cell Biology, Monash University, Clayton, Victoria, 3800, Australia

Background: Epidemiological studies have linked intrauterine growth restriction (IUGR) with an increased risk of cardiovascular disease in adulthood. Studies in several species have found that IUGR is associated with alterations in endothelial and smooth muscle function in peripheral arteries in adulthood. It has also been found that the protein-DNA ratio of the fetal heart is increased following IUGR, suggesting cardiomyocyte hypertrophy. **Aim**: Our aim was to investigate whether small coronary arteries and cardiomyocytes of fetal sheep were affected by IUGR in late gestation. **Methods**: IUGR was induced by umbilico-placental embolisation in catheterised fetal sheep (n=7), by daily injection of microspheres from days 110-130 of gestation. Sham-operated animals (n=7) received vehicle injection. Fetal mean arterial pressure and heart rate were measured at five day intervals throughout the study. At 130 days of gestation, sheep were euthanised and the fetal hearts were collected. Branches of the interventricular branch of the left descending coronary artery (outside diameter ~400μm) were collected and mounted onto wire and pressure myographs. Arteries on the wire myograph were superfused with warm, oxygenated physiological saline (PSS) and both smooth muscle and endothelial function were tested. Arteries on the pressure myograph were superfused with zero Ca^{2+} PSS containing 5mM EGTA and the passive mechanical wall properties were tested. The remaining cardiac tissue was perfused via a Langendorf apparatus with PSS containing collagenase to dissociate cardiomyocytes. Cardiomyocytes from left and right ventricles were separately analysed for area and binucleation. **Results**: The mean arterial pressure was the same in both the IUGR and control groups over the study period. At 130 days of gestation, IUGR fetuses (2.69±0.13 kg) were significantly lighter than controls (3.58±0.29 kg; p=0.02), and the heart-to-body weight ratio was also reduced in the IUGR group (p=0.04). Coronary arteries from IUGR fetuses had enhanced contractions to the vasoconstrictors angiotensin II ($p<0.01$) and the thromboxane analogue, U46619 ($p<0.01$). Endothelium-dependent relaxation was the same in IUGR and control groups. Relaxation to the nitric oxide donor sodium nitroprusside was not different between the groups. The stress-strain relationship for IUGR arteries was shifted to the right compared with controls (p=0.02), indicating increased compliance. Cardiomyocyte binucleation was decreased in both left (p=0.02) and right (p=0.05) ventricles in the IUGR group, suggestive of decreased maturation. Cardiomyocyte area was not different between the groups, indicating that cardiomyocyte hypertrophy did not occur IUGR fetuses. **Conclusions**: IUGR alters the reactivity of small coronary arteries. Increased compliance of these vessels and reduced binucleation of cardiomyocytes suggest that the heart and its vessels are immature in IUGR fetuses. The consequences of these delays in cardiac and coronary maturation could impact on their adaptation to the higher blood pressure experienced following birth.

P2-036

Impairment of Cerebellar Cortex Development of Postnatal Youngs of Albino Rats Maternally Treated with Acrylamide or Supplemented Food Containing Fried Potatoes Chips

*Hassan I. El-Sayyad , Hekmat L. El-Gammal., Lotfy A. Habak. & Heba M. Abdel-Galil Department of Zoology, Mansoura Faculty of Science, Mansoura University, EGYPT. *Prof. Exp.Embryology, Department of Zoology, Mansoura Faculty of Science, Mansoura University, EGYPT.

Acrylamide first received global attention in April 2002 when Swedish researchers reported finding the chemical in fried and oven-baked foods, especially in potato chips and French fries. The present study deals with clarifying the neurotoxic effects on cerebellar cortex development during postnatal development. Eighty fertile male and virgin female rats weighing approximately 120g. body weight (3 female / 1 male) were used in the present work. Fertile males were used for mating and zero date of pregnancy were determined. Three animal groups were arranged.The first group served as control. The second group received daily oral doses of 30 mg acrylamide /kg body weight from day 6th of gestation till parturition as well as during postnatal life till 4 week. The third group, received food supplementation of fried Potatoes chips beside standard diet from day 6th of gestation till parturition as well as during postnatal life till 4 week. The postnatal youngs were sacrificed at parturition as well as 1, 2, 3 & 4 weeks. Criteria of brain growth as well as light, scanning & transmission electron microscopoic observations were carried out on the mentioned ages of both control and experimental groups. The growth pattern of olfactory bulb, cerebral hemisphere, cerebellum & medulla oblongata were markedly retarded in those maternally treated with acrylamide or received food supplementation containing fried potatoes chips. On the other hand, light microscopic observations revealed a considerable reduction of molecular, purkinje cells and internal granular layers of the experimental groups. Different pattern of cell death were detected especially in purkinje cells and neurons of the granular layers of the developing stages maternally receives the previous treatment. TEM observations revealed increased average of apoptic cell death of neuronal cells especially purkinje cells. These was associated with ruptured endoplasmic reticulum , loss arrangement of poly ribosomes , swollen mitochondria with ill differentiated cisternae as well as abnormal pattern structure of Golgi complex.

P2-037

Comparative Effects of Acrylamide and Fried Potatoes Chips Supplementation on Pregnant Mice and Their Prenatal Embryos and Newly Born

* Hassan I. El-Sayyad & Heba A. El-Ghawet; Department of Zoology, Mansoura Faculty of Science, Mansoura University, EGYPT. *Prof. Exp.Embryology, Department of Zoology, Mansoura Faculty of Science, Mansoura University, EGYPT.

Acrylamide first received global attention in April 2002 when Swedish researchers reported finding the chemical in fried and oven-baked foods, especially in potato chips and French fries. The present study deals with illustrating the hazardous effects during pregnancy as well as on embryogenesis. One hundred fertile male ~~and~~ virgin female mice weighing approximately 25g. body weight (2 female / 1 male) were used in the present work. Fertile males were used for mating and zero date of pregnancy were determined. Three animal groups were arranged. The first group served as control. The second group received daily oral doses of 25 g acrylamide /kg body weight from day 6th of gestation till parturition. The third group, received food supplementation of fried Potatoes chips beside standard diet from day 6th of gestation till parturition. The pregnant mothers were sacrificed at 14, 16 & 17 days prenatal as well as at parturition. Histological investigations were carried out in liver, kidney, heart & epiphyseal cartilage of mothers at parturition of both control and experimental groups. The criteria and incidence of morphological and skeletal anomalies were assayed for delivered newly born of both control & experimental groups. The growth pattern during prenatal development till parturition were determined. Histogenesis of affected skeletal regions during prenatal development were illustrated. The present findings reported closely similar histological abnormalities in the mentioned maternal tissues as a result of acrylamide intoxication or during food supplementation of fried potatoes chips. The epiphyseal cartilage exhibited highest drastic alterations of cell structures. Both acrylamide-treatment and fried potatoes chips supplementation increased the rates of aborted mother during the progress of gestation, increased neonatal mortality and reduced the total numbers of newly born comparing with the control. The prenatal fetuses (14, 16 & 17 day-age) as well as delivered newly born maternally-fed on diet containing fried potato chips or received acrylamide-treatment exhibited a marked reduction of their body weight, size & crown-rump length comparing with the control group. Different pattern of congenital malformations were observed at higher rates in those of mothers fed on fried potato chips more than that received acrylamide – treatment. The ossification of axial and appendicular bones were markedly retarded during prenatal growth till parturition and assessed by reduction of length measurement of ossified bones of both mandibular and appendicular regions. Assaying the incidence of missing ossified bones in newly born of the different experimental groups revealed

that the retarded ossified regions restricted mainly in tympanic region, sternbrae, clavicle, ischium, distal phalanges of fore-& hind limbs as well as caudal vertebrae. Highest incidence of missing ossification centers was detected in newly born maternally fed on diet containing fried potato chips comparing with those maternally received acrylamide-treatment. Histogenesis of tympanic region, ribs and humerus and radio-ulna revealed massive retarding skeletal cell elements including chondroblast and osteoblast building cells as a result of acrylamide-treatment or fried potatoes chips supplementation.

P2-038

Effects of Prenatal Lipopolysaccharide Exposure on Epithelial Development and Function in Newborn Rat Intestine Peter J. Giannone,Brandon L. Schanbacher, Charles V. Smith, John A. Bauer, and Kristina M. Reber. Section of Neonatology, Department of Pediatrics, Center for Developmental Pharmacology and Toxicology, Columbus Children's Research Institute, Columbus, OH.

Background: Maternal infection during pregnancy is associated with several neonatal morbidities, including premature delivery. These infections may be symptomatic or subclinical in nature, such as gingivitis. Infants born prematurely often have problems with feeding intolerance and are at risk of developing necrotizing enterocolitis (NEC). Inducible nitric oxide synthase (NOS II) is a mediator of tissue injury at high levels and has been associated with NEC in the newborn intestine. **Objective:** To test the hypothesis that responses to prenatal maternal exposure to LPS alter intestinal epithelial development and function in newborn rats. **Design/Methods:** Timed pregnancy female Sprague-Dawley rats were administered either 2 mg LPS or an equal volume of isotonic saline by intra-peritoneal injection at E15. Dams were allowed to delivery naturally. Pups were weighed and then sacrificed at DOL 0, 3, and 7. At the time of sacrifice, rat pups intestines were removed, divided into 4 sections, and immediately formalin fixed. Morphometric parameters were measured on standard hematoxylin and eosin stained sections using ImagePro software. Images were captured using a 20x objective on a standard upright microscope with an attached digital camera. We measured average villus height (mucosal thickness), submucosal thickness, and muscularis thickness. Measurements were made by tracing the boundary of each transmural region (villus tips, mucosa/submucosa, submucosa/muscularis, and outer muscularis) as illustrated below and averaging the distance between each trace. This provided a straightforward method by which to measure the thickness between these convoluted boundaries. Intraobserver variability with this method was 1.6%. Immunohistochemistry was performed with antibody specific for NOS II and 3-nitrotyrosine (3-NT) on intestinal samples analyzed at each time point. Optical density (OD) was determined and quantified for site-specific regions of intestinal sections. Two-way ANOVA with Bonferroni posttests were performed. **Results:** There were no significant differences in pup weights on DOL 0, 3, or 7 between the prenatal LPS or control groups. However, mucosal thicknesses were significantly less in the distal ileum from pups born to LPS exposed dams on DOL 0 (261.5±77.46 vs. 162.90±19.90), 3 (379.6±61.41 vs. 235.3±43.46), and 7 (452.9±79.72 vs. 416.50±85.23) ($p<0.001$). NOS II protein concentrations in the distal small intestine of the prenatal LPS treatment group was significantly greater than NOS II protein concentrations in the distal villus (0.0777±0.027320 vs. 0.12910±0.042600;$p<0.001$), proximal villus/crypts (0.03082±0.020470 vs. 0.05122+0.038030;$p<0.01$), submucosa (0.02427±0.025800 vs. 0.06720±0.037110;$p<0.001$), and muscularis (0.07577±0.036600 vs. 0.10230±0.043600;$p<0.01$) in the distal small intestine of the control group on DOL 0. There were significant differences observed in NOS II protein concentrations on DOL 3 and 7 in the distal villus and submucosal regions of the distal small intestine noted between groups ($p<0.001$). 3-NT immunostaining was significantly elevated in the prenatal LPS exposed pups in the distal villus on DOL 0 (0.19190±0.033830 vs. 0.24560±0.047470), 3 (0.22410±0.032260 vs. 0.28160±0.032260), and 7 (0.20310±0.032640 vs. 0.25270±0.032640) ($p<0.001$) as well as in the submucosa on DOL 3 (0.13500±0.047210 vs. 0.18130±0.108100;$p<0.001$). Serum FITC measurements were greater in prenatal LPS exposure group (2.413 versus 0.9567) at DOL 14 ($p<0.001$). **Conclusion:** Maternal exposure to LPS during pregnancy alters intestinal growth, barrier function, and regulation of NOS II in the rat newborn intestine. We speculate increased NOS II concentrations in the newborn intestine secondary to maternal infection and/or inflammation may lead to intestinal injury and possibly predispose infants to NEC.

P2-039

Moderate Fetal Alcohol Exposure in Late Gestation Induces White Matter Injury and Increases Apoptosis in the Fetal Brain PA Dalitz[1], ML Cock[1], JR Duncan[2], SM Rees[2], KL Gatford[3], JA Owens[3], R Harding[1]. [1]Dept of Physiology, Monash University, Melbourne, VIC, Australia; [2]Dept of Anatomy & Cell Biol., University of Melbourne, Melbourne, VIC, Australia; Dept of Obstetrics & Gynaecol. University of Adelaide, Adelaide, SA, Australia.

Background: Acute patterns of alcohol exposure in late gestation have been associated with reduced fetal growth and may be particularly harmful to the developing brain. However, fetal neuropathology following such exposures and the underlying mechanisms are largely unknown. **Aims:** To determine the effects of repeated, acute fetal ethanol (EtOH) exposure on the developing cerebral and cerebellar white matter (WM) and hippocampus, and to examine potential mechanisms of fetal cerebral injury and altered fetal growth. **Methods**: EtOH (1g/kg of maternal weight) was administered i.v. to 8 twin-bearing ewes for 1 hour on 3 consecutive days from 116±1 days' gestation (DG; term ~147DG); controls (n=5) received saline. Fetal and maternal blood alcohol concentrations (BAC) and physiological parameters were measured. Autopsy was performed at 120±1DG. Fetal plasma collected prior to and 6h after EtOH infusions, and from 1-4h after infusion after EtOH exposure, was subjected to HPLC to analyse levels of IGF-I, IGF-II and IGF-binding capacity. Plasma collected prior to, and from 1-4h after EtOH, as well as WM collected at autopsy, were analyzed for levels of pro-inflammatory cytokines. Fetal brains were assessed for alterations in gross morphology, astrocytes, microglia/macrophages, axons, oligodendrocytes and apoptosis. The incidence of astrogliosis (GFAP+) and the density of apoptotic cells (TUNEL+) and oligodendrocytes (CNPase+) were examined within the peri-ventricular-, deep- and sub-cortical WM in the frontal, parietal, temporal and occipital lobes. Apoptotic cells were also quantified in the hippocampus and cerebellar deep WM. Fetal brain tissue was analysed for levels of lipid hydroperoxide (LPO) as an index of oxidative stress. **Results:** Maternal and fetal BAC peaked at 0.11±0.01 g/dL 1h after the EtOH infusions. Fetuses did not become hypoxemic or acidemic. At autopsy, mean body weights of EtOH fetuses were ~400g lower than controls ($p=0.02$) indicative of growth arrest. Fetal plasma IGF-II tended ($p=0.099$) to be reduced 54h after EtOH; IGF-I was unchanged. In 4/8 EtOH fetuses, areas of subcortical WM injury were present in all lobes: damage was substantial in 2 fetuses and modest in 2 fetuses. Injury was characterised by axonal disruption and infiltrating microglia/macrophages. Apoptotic cells and astrocytes were significantly increased throughout the cerebral WM of EtOH fetuses compared to controls ($p\leq0.05$). In 2/8 EtOH fetuses, cerebellar WM gliosis was observed. Apoptotic cells tended to be more numerous in the deep cerebellar WM of EtOH fetuses in comparison to controls ($p=0.06$) but there was no difference between groups in the hippocampus. No brain injury was observed in control fetuses. Fetal cerebral WM and plasma pro-inflammatory cytokine levels were not altered by EtOH exposure. LPO tended to be elevated in cerebral WM, corpus callosum, hippocampus, brainstem, thalamus and spinal cord of EtOH fetuses in comparison to controls. **Conclusions:** Episodic exposure of the immature fetus to modest levels of EtOH can result in overt brain injury and an increase in WM apoptosis and astrogliosis. Fetal neuropathology following EtOH exposure could involve oxidative stress. Altered fetal IGF levels may contribute to alterations in fetal growth following prenatal alcohol exposure.

P2-040

Cigarette Smoke Exposure During Pregnancy Leads to Increased Systemic Arterial Blood Pressure and Attenuated Coronary Artery Relaxation Kathleen J. Lumb, Christopher Triggle, Xiolan Zhou, Jonathan Pendlebury and Shabih U. Hasan. Department of Pediatrics and Institute of Maternal and Child Health, The University of Calgary, Calgary, Alberta, Canada

Background: Intrauterine development is a critical period for the correct patterning and organization of fetal tissues. Cigarette smoke (CS) exposure during pregnancy is the most common exogenous chemical insult to the developing fetus and may be a factor in the occurrence of adult disease including diabetes, hypertension and ischemic heart disease. **Methods and Experimental Design:** We investigated the effects of perinatal CS exposure on vascular contractile and relaxation responses, and arterial blood pressure in adult Wistar-Kyoto rats. At 100 days of age, male offspring were implanted with a femoral artery catheter attached to a radiotelemetry device for the measurement of blood pressure, heart rate and activity. This allowed us to monitor the animals from a remote location, thus avoiding erroneous readings due to handling stress. No sooner than 10 days after surgery, cardiovascular variables were recorded every 5 min for 24 hours on day 110, 120, and 130. Dose responses (log EC50) to the contractile agents 9,11-dideoxy-11a, 9a-epoxymethano-prostaglananin-F2a (U46619) for coronary artery (CA) and phenylephrine for the mesenteric artery (MA) and aorta (Ao), and the endothelium-dependent vasodilator acetylcholine (Ach) were obtained. Subsequently, the effects of indomethacin, L-NAME, the soluble guanylyl cyclase inhibitor (ODQ), apamin, charybdotoxin and K^+ on the Ach mediated vascular relaxation were investigated. Random effects generalized least square regression analyses were performed to investigate the independent effects of age and treatment on systemic arterial blood pressures and heart rate. **Results:** Our data suggest that as compared with the control group, the CA of CS exposed animals demonstrated a decreased relaxation response to Ach and that addition of L-NAME completely inhibited the Ach mediated relaxation. The arterial blood pressure, measured using radiotelemetry devices, was also significantly higher in CS exposed group. Furthermore, the changes in vascular responsiveness were independent of nutritional status of the animals. **Conclusions:** We present novel and exciting evidence that prenatal CS exposure has long-term deleterious effects on vascular endothelial and/or smooth muscle integrity. It is likely that CS exerts such aberrant vascular changes during fetal angiogenesis. Our data are not only important for raising public awareness but may also provide a basis for future gene targeting and therapeutic interventions in humans.

P2-041

Chronic Prenatal Ethanol Exposure Increases Glucocorticoid-Induced Stimulated Glutamate Release in the Hippocampus of the Near-Term Fetal Guinea Pig Umar Iqbal, James F. Brien, and James N. Reynolds; Department of Pharmacology & Toxicology and the Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada K7L 3N6

Background: Exposure to high cortisol concentration ([cortisol]) can injure the developing brain, possibly via an excitotoxic mechanism involving glutamate (Glu), an excitatory neuroactive amino acid. Chronic prenatal ethanol exposure (CPEE) also injures the developing brain via multi-faceted mechanisms. Our current research program is based on the novel postulate that there is an important interaction between

CPEE and elevated fetal [cortisol], which determines the extent of injury in the hippocampus, a susceptible region of the developing brain. **Objective**: The present study tested the hypothesis that CPEE increases sensitivity to glucocorticoid-induced Glu release in the near-term fetal hippocampus. **Methods**: Timed pregnant Dunkin-Hartley-strain guinea pigs were treated with ethanol (4g/kg maternal body weight/day) or isocaloric-sucrose/pair-feeding through gestation and were euthanized at gestational day (GD) 63 (term, about GD 68), 1 h after the daily ethanol dose. Basal and electrically stimulated efflux of Glu and GABA, in the presence or absence of dexamethasone (DEX), a selective glucocorticoid-receptor agonist, was determined *ex vivo* in fetal transverse hippocampal slices, using HPLC-fluorescence-detection analysis of the derivatized neuroactive amino acids. **Results**: In the near-term fetal hippocampus, there was a [DEX]-dependent increase in basal release of Glu, but not GABA, and there was no effect of CPEE. There was a selective [DEX]-dependent effect on stimulated release of Glu, but not GABA. A low [DEX] (0.3 μM) suppressed stimulated Glu release, and there was no effect of CPEE. In contrast, for CPEE, a high [DEX] (3.0 μM) increased stimulated Glu release compared with the nutritional control. **Conclusions**: These data indicate that CPEE alters glucocorticoid regulation of stimulated Glu release in the near-term fetal hippocampus. This effect of CPEE may predispose the fetal hippocampus to Glu-mediated excitotoxic neuronal cell death in late gestation, when fetal [cortisol] is increased in preparation for birth. (Supported by Canadian Institutes of Health Research)

P2-042

Heme Oxygenase Activity in Fetal and Adult Ovine Tissue: Effects of High-altitude-induced, Long-term Hypoxemia Robert T. Kinobe, Jonathan M. Soong, James F. Brien, Lawrence D. Longo and Kanji Nakatsu; Department of Pharmacology and Toxicology, Queen's University, Kingston, ON, K7L 3N6 Canada and Center for Perinatal Biology, Loma Linda University, School of Medicine, Loma Linda CA 92350 USA

Background: Heme oxygenases (HO, EC 1.14.99.3) are involved in the biotransformation of heme to biliverdin/bilirubin, ferrous iron and carbon monoxide (CO). It is known that products of heme catabolism play a role in the regulation of many physiological processes including vascular tone, inflammatory responses and central nervous system function. The HO system is also involved in cytoprotection against oxidative stress. In mammals, there are two catalytically functional HO isozymes, HO-1 (inducible) and HO-2 (constitutive). The expression and function of HO and particularly HO-1, is regulated by an array of physiological and non-physiological conditions including the exposure to heavy metals, ionizing radiation and acute hypoxemia. As relatively little is known of the HO response to prolonged hypoxemia in whole animals other than small laboratory rodents, we examined the effect of long-term hypoxemia on total HO activity in fetal and adult ovine tissue. **Methods**: Sheep were maintained at high altitude (3,820 m) after which the following tissues were harvested from near term fetal and non-pregnant adult ewes for *in vitro* measurement of HO activity: left heart ventricle, renal papilla, lung apex, pulmonary artery, carotid artery, mesenteric artery, placental cotyledon, spleen and brain frontal cortex. Relative HO-1 and HO-2 protein expression was quantified by sodium dodecyl sulfate polyacrylamide gel electrophoresis and HO activity was measured by quantitation of CO using gas chromatography. **Results and conclusions**: HO activity was detected in all the tissues examined but there were no significant differences between hypoxic fetal and adult sheep compared to their normoxic controls. Fetal heart HO activities were higher than those of adult tissue ($P < 0.05$), while adult spleen HO activity was significantly higher than that of fetal tissue ($P < 0.05$). These data indicate that high-altitude-induced hypoxemia does not have a persistent effect on HO activity in ovine tissues. Also except for the spleen where there is a high expression of HO-1, under normal conditions, tissue HO activity is correlated with the expression of HO-2, the constitutive isozyme. In some body tissues such as the heart and the spleen, HO expression and enzymatic activity may be developmentally regulated. (This work was supported by Canadian Institutes of Health Research Grant MOP 64305 and National Institutes of Health Research Grant PO1-HD-31226).

P2-047

Later Life Correlated Brain/CNS and CVS Effects of Prenatal Environmental Exposure Sergiy Volovik, Konstantin Loganovsky, Tetyana Loganovsky, Dimitry Bazyka, Volodymyr Bebeshko Duke University, Durham, North Carolina 27708-0408 USA Research Centre for Radiation Medicine, Kyiv 04210 Ukraine

Objective: To interpret and conceptualize coordinated response in brain/CNS and cardiovascular system to environmental stress (ionizing radiation) exposure for persons born between April 26, 1986 and February 26, 1987 from pregnant evacuated to Kiev from Exclusion Zone (Prypiat) after Chernobyl accident. **Methods:** Analysis of the individual effective fetal, brain, and thyroid dose reconstruction in utero, systems changes in structural / functional similarity, growth and transcriptions factors, free-radical/oxidative biomarkers, IQ discrepancies, and dynamic EEG and ECG data for persons with neuropsychiatric disorders, neurodegeneration, and cognitive impairment from Chernobyl Exclusion Zone and matched health controls. **Results:** The coordinated alterations in the systems structural, growth and transcription factors, ROS/RNS production / elimination balance (superoxide-nitric oxide-peroxynitrite equilibrium), IQ deterioration, and characteristic correlated EEG and ECG patterns depending on matching dose were revealed. **Conclusions:** Analysis of findings leads to summing up that such self-consistent response in brain/CNS and cardiovascular system to ionizing radiation stress is a consequence of perturbation of free-radical ROS/RNS/RSS redox dynamic homeostasis, perturbed redox signaling networks, concomitant alterations in redox genes expression and transcription, impaired redox control of metabolic energy budget, and hemispheric dominance.

Developmental Plasticity

P2-048

Strain Differences in Body Weight, Activity, and Anxiety in Mice Associated with Variations in Maternal Care Frances A. Champagne, James P. Curley, Eric B. Keverne, and Patrick Bateson, Sub-Department of Animal Behaviour, University of Cambridge, High Street Madingley Cambridge CB3 8AA

Background: Recent evidence suggests that strain differences in physiology and behavior in mice are mediated in part by maternal pre-natal and post-natal environments. Thus, phenotypes that were once considered to be genetic in origin may be the product of altered gene expression induced by variations in maternal care. The role of maternal licking/grooming behavior in generating both within and between strain differences in phenotype has previously been demonstrated in rats. Offspring born to mothers who show elevated levels of licking/grooming appear less anxious in novel situations and have an attenuated HPA response to stress. Though phenotypic variation between strains of mice has been well documented, the study of the role of early rearing experience in generating these differences has yet to be established. **Methods**: We conducted detailed observation of maternal care in 129Sv and C57BL/6J (B6) adult female mice. Females were mated and singly housed a week prior to parturition. On the day of birth (P_0), mother and pups were weighed and a test of pup retrieval was conducted. Following testing, females were left undisturbed for 1 week in Plexiglas cages and mother-pup interactions were observed. Maternal and pup behavior were also observed in the week prior to weaning. At weaning, pups were weighed and housed 5/cage until day 70, when they were tested in an open-field apparatus and measures of activity and exploration were taken. **Results**: Though no differences in latency to maternal behavior were determined on the retrieval test, frequency of licking/grooming (LG) and nursing over the first week postpartum (P_1-P_6) differ significantly between the strains. B6 females display a higher degree of LG (M=10.3%) compared to 129Sv females (M=4.2%) and spent less time nursing pups (M=60% *vs.* M=70%). These strain differences in maternal care were also observed in the week prior to weaning (P_{21}-P_{27}). Litter size and average pup weight at P_0 did not differ between strains, however, by weaning (P_{28}) 129Sv pups weighed significantly more than B6 pups. Home cage observations of activity levels of pre-weaning pups and adult offspring of 129Sv and B6 mothers indicate higher levels of activity in the B6 strain. Differences in activity and anxiety were also observed in the open-field test. Thus, the high levels of nursing and low levels of LG characteristic of 129Sv mothers predicts a higher juvenile body weight, lower activity levels, and higher anxiety in offspring, whereas the low levels of nursing and high levels of LG typical of B6 mothers predicts a lower juvenile body weight, higher activity levels, and lower anxiety in offspring. Preliminary data from reciprocal crosses of these two strains confirms the association between these phenotypic outcomes and these variations in maternal care. **Conclusions**: Maternal care serves as an important regulator of gene expression and behavior. This has been demonstrated experimentally in rats and the present study confirms the association of maternal care and offspring phenotype in mice. We propose that differences in frequency of nursing activity during the post-partum and pre-weaning periods is an important cue for determining levels of activity whereas, LG serves as a cue for moderating levels of anxiety. These findings have implications for our understanding of the role of maternal care in developmental outcomes and for genetic studies using particular background strains in transgenic and targeted knockout manipulations.

P2-049

Sex Differences in Exploration and Anxiety in Offspring and Grand-offspring of Mice Impaired in Maternal Care, James P. Curley, Frances A. Champagne, Patrick Bateson & Eric B Keverne, Sub-Department of Animal Behaviour, University of Cambridge, CB3 8AA, UK

Background: Developing offspring may modify both their physiology and behavior in response to the early maternal environment of both the pre- and post-natal phases. Although these changes are often pathological and disruptive to development, they may be adaptive to the organism. Under such circumstances, the cues received by offspring via the mother act as a barometer for the external physical and social environment, enabling individuals to facultatively alter their development such that they will be better prepared for the post-weaning period. While there is increasing evidence for these effects in physiological systems, such as with thrifty phenotype metabolic adaptations to *in utero* caloric and nutrient restriction, adaptive behavioral developmental responses are little studied. Nevertheless, recent work in rodent models has revealed that individuals do epigenetically alter their behavior, particularly levels of anxiety, in response to different maternal environments. To study potential adaptive epigenetic changes there is therefore a need to establish relevant animal models of developmental plasticity, investigating the behavioral development of offspring and grand-offspring of both sexes exposed to different pre- and post-natal maternal environments. **Methods:** Female 129Sv mice lacking the paternally expressed gene *Peg3* suffer several impairments in maternal care during both the pre- and post-natal phases, including the *in utero* transfer of resources, milk let-down, pup retrieval, nestbuilding and crouching over the pups. Significantly, due to the expression pattern of this gene, mutant mothers mated with wild type males give birth to wild type offspring, allowing us to investigate the effect of impaired maternal care on the anxiety and exploratory behavior of normal adult offspring. Moreover, by mating the daughters

of mutant mothers with wild type males, we are able to assess the transgenerational non-genomic transmission of behavioral phenotypes. **Results:** Both wild type sons and daughters of mutant mothers are growth retarded throughout lactation and post-weaning at day 28pn. By puberty, females born to mutant mothers catch up in growth to wild types and are normal weight throughout adulthood. Conversely, sons of mutant mothers continue to be growth retarded at puberty and beyond. This loss in sexual dimorphism in body weight of offspring of mutant mothers is also observed in behavior. Daughters of mutant mothers are more anxious and less exploratory than wild types, but there is no difference in behavior between sons of wild type and mutant mothers. Moreover, like their mothers, these daughters are impaired in their ability to retrieve pups immediately postpartum in a test of maternal behavior. This may be indicative of a 'high stress' phenotype that is also associated with lower exploration of the novel object and open-field. Significantly, this impairment is also observed in the female, but not the male, offspring of daughters born to mutant mothers. **Conclusions:** This work demonstrates that behavioral phenotypes in mice may be transmitted via a non-genomic mechanism and that there may be sex differences in the responsiveness to early life experiences. In particular, daughters may be more sensitive to deteriorations in the quality of the maternal (and therefore external) environment than sons, investing heavily in growth post-weaning to ensure that they can enter puberty. This short-term benefit may come at the longer-term cost of impaired behavioral development for themselves and their offspring.

P2-050

Growth and Gene Expression of Components of the Renin Angiotensin System in Offspring of Rabbits with Chronic Maternal Hypertension

Kate M Denton[1], Karen Moritz[2], Rebecca L Flower[1], E Marelyn Winour[1] Devaki Maduwegedera[1], [1]Department of Physiology and [2]Department of Anatomy, Monash University, Victoria, 3800, Australia.

Introduction: Chronic hypertension complicates ~5% of pregnancies, an incidence expected to rise given the trend for women to have children later. It has been suggested that permanent alterations in fetal development due to adverse conditions such as chronic hypertension 'program' the fetus. We have shown using a two-kidney, one-wrap (2K-1W; renin dependent) model of hypertension that adult female offspring of hypertensive mothers have increased blood pressure. To better understand the developmental process that may pre-dispose offspring of chronically hypertensive mothers to develop hypertension in later life, the aims of this study were to characterise growth and gene expression of the renal renin angiotensin system (RAS) during development and to determine glomerular number. **Methods**: Three groups were studied, 2K-1W (n=18); two kidney, two wrap (2K-2W; renin independent, n=21) hypertensive, and sham operated (n=20) normotensive mothers. Biometric measurements of offspring were made and kidneys collected for determination of gene expression levels at gestational age (GA) 21, 28, birth, and 5 weeks of age. Expression of AT_1, AT_2 and renin was studied using real-time PCR. Metanephric development begins at GA11.5 in the rabbit and is complete by postnatal day 21, after which glomerular number was determined stereologically **Results**: Body and kidney weights were similar in the groups at GA21 and 28. However, 2K-1W offspring demonstrated 20% greater birth weights and 30% greater kidney weights compared to sham and 2K-2W offspring. At 5 weeks there was no significant difference in body or organ weights between groups. Gene expression results from GA 21 and birth (Table) indicate differences in the ratio of AT_1 to AT_2 receptors in the kidneys of 2K-1W offspring at birth, and 2K-2W offspring in early gestation. Preliminary data suggest that 2K-2W offspring have more but smaller glomeruli (P=0.06) **Conclusion**: Offspring of 2K-1W and 2K-2W hypertensive mothers show alterations in the key receptors of the RAS. Given the significant role of the RAS in kidney development and salt and fluid homeostasis, these changes may contribute to the development of adult hypertension in offspring of hypertensive mothers.

P2-051

Umbilical Liver Blood Flow, Degree of Ductus Venosus Shunting and Their Relationship to Cerebral Circulation in the Normally Growing Fetus

Cathrine Ebbing[1], Svein Rasmussen[1], Keith Godfrey[2], Mark Hanson[2] and Torvid Kiserud[1] [1]Institute of Clinical Medicine, Department of Obstetrics and Gynaecology, University of Bergen, Norway, [2]Division of Developmental Origins of Health and Disease, University of Southampton, Southampton, UK

Background: Experimental studies suggest that the umbilical blood flow to the fetal liver is an intra-uterine growth determinant. On the other hand side, the ductus venosus (DV) directs a proportion of the umbilical venous return directly to the heart thus bypassing the fetal liver, particularly during fetal hypovolemia and hypoxemia. It is possible that this distributional antagonism between liver and brain is reflected in the variation of developmental adaptation across the normal population. We therefore tested the hypothesis that there is an interdependency between the umbilical liver perfusion, degree of DV shunting and blood flow velocity patterns in the middle cerebral artery (MCA) in normally growing fetuses. **Methods:** 104 healthy women with low-risk pregnancies were recruited after written consent to a longitudinal observational study approved by the Regional Committee of Medical Research Ethics. The women were examined 1-4 times at 20-40 weeks of gestation using ultrasound imaging and Doppler techniques. The peak systolic velocity (PSV) and pulsatility index (PI) in the MCA have previously been shown to reflect circulatory brain sparing effect, and were thus used in the present study to reflect degree of circulatory priority. Diameter and time-averaged maximum velocity (TaVmax) in the DV and UV were determined to calculate blood flow to the liver and DV and the fraction of shunting through the DV. Since the data were dependent of gestational age, we expressed the observations as standard deviation scores (z-scores), which were calculated using multilevel polynomial regression. Linear regression analyses were used to determine whether blood flow parameters of the fetal brain and liver and DV were interdependent. Quartiles were used to identify any threshold effects in an ANOVA analysis. **Results:** 331 sets of observations (215 before and 116 after 32 weeks) showed that, overall, PI and PSV in the MCA varied independently compared to TaVmax and degree of shunting in the DV, and UV blood flow to the liver (Table). However, in the subgroup before week 32, DV shunting was associated with increased MCA PI (overall p=0.03), mainly due to the effect seen in the lowest quartile of shunting (mean 0.34 z-score, 95%CI 0.01-0.67, overall p=0.003 when analysed separately).

	Slope	95% confidence interval	P value
MCA PI vs. DV shunting	-0.12	-0.26: 0.02	0.09
MCA PSV vs. DV shunting	0.008	-0.14: 0.16	0.91
MCA PI vs. TaVmax of DV	0.026	-0.09: 0.14	0.66
MCA PI vs. UV-DV (UV liver flow)	0.071	-0.07: 0.21	0.33
MCA PSV vs. UV-DV (UV liver flow)	0.107	-0.03: 0.24	0.11

Conclusions: During normal fetal growth the liver and brain circulation seems to be largely independently regulated, but with some sign of interdependency in the extreme group, in our study before 32 weeks, and in a previous study, at 36 weeks, both indicating threshold effects.

P2-052

NCX and SERCA Genes are Not Regulated by Hemodynamic Stress in the Fetal Sheep Heart

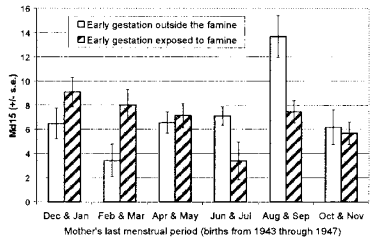
Sonnet S. Jonker, Tara Karamlou, J. Job Faber, Debra F. Anderson, George D. Giraud and Kent L. Thornburg; Departments of Physiology and Pharmacology, Medicine (Cardiology), and the Heart Research Center, Oregon Health & Science University, and the Portland VA Medical Center, Portland, OR, 97239 USA

The storage, release and uptake of calcium within cardiac myocytes regulate the force of contraction and the rate and extent of myocardial relaxation. The development of these mechanisms during fetal life permits more efficient calcium cycling due to increased use of intracellular calcium stores. The sarcolemmal sodium-calcium exchanger (NCX) plays a more prominent role in the immature myocardium than does the sarco(endo)plasmic reticulum ATPase (SERCA). In some adult hearts a pathological response to chronic hemodynamic overload is re-expression of high levels of NCX and decreased expression of SERCA; this accelerates the progression of disease because the heart becomes less efficient in using ATP to pump calcium. We hypothesized that chronic cardiovascular load of fetal sheep would increase the expression of NCX and decrease that of SERCA. If maintained, such changes would increase the vulnerability of the myocardium for adult onset heart failure. **Methods:** To test this hypothesis, we obtained hearts from three groups of late-term fetuses subjected to chronic hemodynamic stress and their controls: Group A were volume-loaded for 7 days via an arterio-venous fistula created between the carotid artery and jugular vein (experimental and control each n=5). Group B were pressure-loaded for 10 days via an inflatable occluder on the post-ductal aorta (each n=6). Group C received intravascular plasma infusions for 8 days to impose a volume and pressure load (each n =6). Hearts were also obtained from 95-day fetuses (n=5), 135-day fetuses (n=5), neonates (n=4) and non-pregnant ewes (n=5) to determine the developmental regulation of these genes. Northern blot analysis was performed and blots were quantitated using NIH ImageJ. Results were normalized to the expression in control RV. **Results:** The relative expression of the NCX /SERCA ratio decreased dramatically during development (figure). **Group A:** Pulmonary artery flow increased by nearly 3-fold over 7 days of loading (385±137ml/min to 1,003±393ml/min), and RV mass increased (control 7.6±3.0g, experimental 8.6±1.0g). The ratio of NCX to SERCA did not change (LV 1.1±0.3, RV 1.2±0.7). **Group B:** Arterial pressure increased by almost 20% over 10 days of load from 48.3±6.4mmHg to 57.4±6.0mmHg. The ratio of NCX to SERCA did not change (LV 1.1±0.2, RV 1.0±0.1). **Group C:** Over 8 days of load, arterial pressure increased from 42±3mmHg to 62±5mmHg and venous pressure increased from 2.6±0.8mmHg to 4.1±1.5mmHg. Cardiac mass increased from 27±7g in controls to 37±4g in loaded fetuses. The ratio of NCX to SERCA did not change (LV 1.0±0.1, RV 1.0±0.0). **Conclusions:** NCX and SERCA undergo dramatic changes in expression during development. Although chronic hemodynamic stress can induce cardiac hypertrophy, the late-gestation fetal heart is resistant to the regulation of these genes by hemodynamic stress. Thus, it is unlikely that altered expression of cardiac calcium transport genes by hemodynamic stress in the fetus programs the susceptibility of the adult heart to disease.

P2-053

Early Gestational Environment is Associated with a Fingerprint Ridge-count Gradient: The Dutch Famine Study Henry S. Kahn, Mariaelisa Graff, Aryeh D. Stein, Patricia A. Zybert, L.H. Lumey; National Center for Chronic Disease Prevention and Health Promotion (CDC), *Atlanta, GA 30341 USA;* Rollins School of Public Health, Emory University, Atlanta, GA; Mailman School of Public Health, Columbia University, New York, NY.

Background: Human fingerprints, including each fingertip's ridge count (RC), achieve their permanent configuration before the 20th week of gestation. Each RC approximates the relative size of the early fetal finger. Correlations between fingerprints and the early intra-uterine environment have not previously been demonstrated. We hypothesized that a gradient of RCs (the difference between RCs on the 1st and 5th fingertips [d15]) would reflect the environment's cephalo-caudal (anterior-posterior) effects on limb-bud morphogens. **Methods**: From birth records in three Dutch cities we traced 569 offspring (born in 1943-1947) who provided left- and right-hand fingerprints from which the mean d15 (Md15) could be counted. We analyzed Md15 according to the calendar month of mother's last menstrual period. We used the date of the last menstrual period to distinguish offspring whose early gestation occurred during the wartime famine from November 26, 1944, through May 12, 1945 (famine exposed, N=318) from those whose early gestation occurred outside the famine period (N=251). **Results**: For men and women without famine exposure the fingerprints showed decreases in Md15 for conceptions occurring in late winter relative to those in late summer (winter-to-summer increment = 10.3, adjusted for sex and maternal smoking; p<0.0001). This seasonal effect reflected a summer RC increment on the 1st finger (+9.7, p=0.0006); there was no summer RC increment on the 5th finger (-0.4, p=0.8). The seasonal effect was not evident among offspring exposed to famine in early gestation. Conceptions occurring during the most severe famine (February-March 1945) had an Md15 increment of +4.6 relative to those occurring in the same winter months outside the famine period (p=0.013).



Conclusions: Both season of year and exposure to famine were associated with the fingertip RC gradient. We did not anticipate the seasonal environmental effect seen in the absence of famine, and its biological mechanisms remain to be investigated. The "natural experiment" of the Dutch famine may guide future studies of associations between fingertip RC gradients and maternal diet or other circumstances of early pregnancy. [The findings and conclusions in this report have not been formally disseminated by the CDC and should not be construed to represent any agency determination or policy.]

P2-054

Umbilical and Portal Venous Volume Blood Flow in the 2nd and 3d Trimester – A Longitudinal Study in Low-Risk Pregnancies Jörg Kessler[1], Svein Rasmussen[1], Keith Godfrey[2], Mark Hanson[2], Torvid Kiserud[1]; National Health Council, and [1]Department of Obstetrics and Gynaecology, Institute of Clinical Medicine, University of Bergen, 5021 Bergen, Norway, and [2]Division of Developmental Origins of Health and Disease, University of Southampton, Southampton, UK

Background: The fetal liver has a regulatory function in growth and development in utero. Animal experiments showed that IGF 1 secretion and DNA synthesis in peripheral tissues are directly dependent on the liver perfusion. Studies in the human has shown that maternal factors such as body composition and diet influence the fetal liver perfusion in late gestation. Since little is known of the development of the portal venous circulation in the human fetus, we aimed at assessing the developmental changes during the second half of pregnancy. **Methods:** 89 healthy pregnant women were recruited to a longitudinal study starting at 20-22 weeks of gestation and including four ultrasound examinations every four weeks. A Sonos 7500 ultrasound machine (Fa. Philips) was used to identify the intraabdominal portion of the umbilical vein (UV), the ductus venosus (DV), and the main stem of the portal vein (PV). The inner diameter (D) of these vessels was measured 3-5 times with a perpendicular insonation to the vessel wall. Blood flow velocities (V) were measured using pulsed Doppler with an angle of insonation as low as possible and always less than 30°. Volume blood flow was calculated as: $Q=p(D/2)^2hV$ (*h*: velocity profile h=0,7 for the ductus venosus, h=0,5 for the other veins). The umbilical volume blood flow to the liver ($Q_{UV\text{-}liver}$) was calculated as: $Q_{UV\text{-}liver} = Q_{UV} - Q_{DV}$. The total venous blood supply of the fetal liver was calculated as: $Q_{liver} = Q_{UV\text{-}liver} + Q_{PV}$. The volume blood flow was normalized for estimated fetal weight based on birth weight percentiles. Statistical analysis included regression analysis and the use of a multilevel model. **Results:** The venous blood supply to the fetal liver increased throughout pregnancy, but, when normalized for fetal weight, showed a continuous decrease. The contribution of portal venous blood was at average 11-12% of the total venous supply until 30 weeks and increased thereafter to reach 20% at term. Interestingly, the variation was more pronounced near term, and in some fetuses, the portal vein fraction constituted as much as half of the total venous blood flow to the liver. **Conclusion:** The portal contribution of blood to the fetal liver is small during the second half of pregancy, but almost doubles during the third trimester while the umbilical contribution reduces correspondingly. In some fetuses, the portal contribution can mount to 50% near term, probably signifying an upregulation of splanchnic contribution and vaso-regulation.

P2-055

Effects of Maternal Hypoxia During the Preimplantion Period on Fetal, Placental and Postnatal Development in the Mouse Karen L Kind, Rebecca L Kelley and Jeremy G Thompson. Research Centre for Reproductive Health, Department of Obstetrics and Gynaecology, University of Adelaide, Adelaide, South Australia, 5005.

Background. Oxygen is an important component of the preimplantation embryonic environment. Reducing the oxygen concentration used during in vitro culture of mouse embryos perturbs embryonic gene expression (1) and subsequent fetal development. Expression of genes induced by exposure to low oxygen, including glucose transporters and vascular endothelial growth factor, is increased in mouse blastocysts cultured under 2% oxygen during the post-compaction period, compared to embryos cultured under 7% or 20% oxygen or developed in vivo (1). The weight of fetal mice developed from embryos cultured at 2% oxygen is reduced at day 18 of pregnancy. Whether factors that alter maternal or uterine oxygen levels during the preimplantation period can also impact on embryonic or fetal development is not certain. The aim of this study was to determine the effect of exposing mouse embryos to maternal hypoxia during the morula to blastocyst stage on subsequent fetal and placental development. **Methods**. Adult female CBA x C57Bl6 F1 mice were mated with males of the same strain. The mice were placed into a clear box at 0600 hours on day 4 of pregnancy (~77 hours post-mating). The box was then infused with 10% oxygen, balance nitrogen (hypoxia) or air (control) for 6 hours. Embryos at this time were at the compact morula to blastocyst stage. At day 18 of pregnancy (term = 19 days) fetal and placental weights were measured (control: n=11 litters, hypoxia: n=10 litters). Further mice were allowed to deliver (control: n=7, hypoxic, n=7) and pup weight was measured 24 hours following birth, and at weekly intervals until 4 weeks of age. **Results**. Maternal hypoxia did not alter litter size at day 18 of pregnancy (control: 8.4 ± 0.4, n=11 vs hypoxia: 7.8 ± 0.5, n=10, mean ± sem), or at birth (control: 8.7 ± 0.4, n=7 vs hypoxia: 9.1 ± 0.6, n=7). Fetal weight at day 18 of pregnancy was reduced (-6%) by preimplantation exposure to hypoxia (control: 910.7 ± 11.2 mg vs hypoxia: 858.3 ± 7.3 mg, p<0.05). Maternal hypoxia did not alter placental weight (control: 74.2 ± 1.1 mg vs hypoxia: 73.5 ± 1.0 mg) or fetal:placental weight ratio (control: 12.6 ± 0.3 vs hypoxia: 11.8 ± 0.2). Weight of pups measured 24 hour after birth was not altered by exposure to maternal hypoxia (control: 1.44 ± 0.01 g vs hypoxic: 1.47 ± 0.02 g). No differences in body weight were evident prior to or at weaning at 21 days of age. However, male offspring born to mothers exposed to hypoxia had lighter weights at 28 days of age (control: 17.3 ± 0.2 g, n=37 vs. hypoxic: 16.3 ± 0.3 g, n=25, p<0.05). **Conclusion**. Maternal hypoxia for six hours during the preimplantation period in the mouse reduced fetal weight in late gestation, but did not alter birthweight. Effects on postnatal growth were evident in male offspring at 4 weeks of age and ongoing monitoring will determine whether these effects persist. This study supports the suggestion that oxygen is an important component of the embryonic environment, that if varied may have consequences for fetal development. (1) Kind KL, Collett RA, Harvey AJ, Thompson JG. 2005. Oxygen-regulated expression of GLUT-1, GLUT-3 and VEGF in the mouse blastocyst. Mol Reprod Dev 70, 37-44.

P2-056

Maternal Hemoglobin Concentration in Pregnancy and Offspring Size at Birth Suhas R. Otiv[1], Chittaranjan S. Yajnik[1], Swapna S. Deshpande[1], Kurus J. Coyaji[1], Dattatray S Bhat[1], Caroline HD Fall[2]; 1Diabetes Unit and Dept of Obstetrics & Gynecology, KEM Hospital & Research Centre, Pune, India. 2.MRC Environmental Epidemiology Unit, Southampton, UK.

Background. It is widely believed that anemia contributes to low birth weight by causing fetal growth restriction and premature birth. However the evidence supporting this association is equivocal and flawed by methodological problems. No study has systematically accounted for confounding factors that affect the birth weight – gestational age, maternal size, parity, nutritional intake and physical activity in pregnancy. In the Pune Maternal Nutrition Study, we studied the association between maternal hemoglobin concentration and offspring size at birth. The careful design of the study allows us to adjust for the effect of these confounding factors. **Methods.** 2466 women of childbearing age in 6 villages near Pune were visited at home 3 monthly to assess their anthropometry and menstrual dates. Women were enrolled if a singleton pregnancy was detected on USG at 18 weeks. 814 mothers were evaluated at 18 and 28 weeks gestation for measurement of: dietary history, physical activity, blood pressure, anthropometry, Coulter based haemogram, ferritin, vitamin C, red cell folate, and other biochemical parameters. The baby's weight, length and placental weight were measured at birth. **Results**. Mean haemoglobin concentration at 18 wks gestation was 11.5 ± 1.4 g% and at 28 wks gestation it was 11.1 ± 1.5 g%. At 18 wks 29% and at 28 wks 42% women were anemic (Hb <11g%). Moderate anemia (Hb 70 – 100 g/L) was present in 12.3% women at 18 weeks and in 20.1% women at 28 weeks. Severe anemia (Hb < 70 g/L) was present in less than 1% women. Two third of anemic women had microcytic anemia and less than 1% had macrocytic anemia. Lower maternal hemoglobin concentration predicted higher birth weight (r= -0.09, p<0.05), as did lower MCV (r= -0.08, p<0.04), lower MCH (r= -0.10, p<0.008), lower serum ferritin (r= -0.07, p<0.05) and lower serum albumin concentration (r= -0.07, p<0.04) at

28 weeks gestation. Lower maternal haemoglobin concentration also predicted heavier placenta ($p<0.01$). Other hematological measures hematocrit, red cell count, MCHC or platelet count at 28 weeks did not predict birth weight. The birth weight was not associated with any of the hematological measures at 18 weeks gestation nor to the change between 18-28 weeks gestation. Multivariate analysis confirmed an independent inverse relation between maternal hemoglobin concentration at 28 weeks and offspring birth weight (standardized ß= -0.08, $p=0.02$), adjusted for gestation age, baby's gender, maternal size, parity and serum albumin concentration and red cell folate concentration. **Conclusion:** Lower maternal hemoglobin concentration independently predicted higher offspring birth weight and higher placental weight. Lower maternal haemoglobin concentration may represent haemodilution and vascular dilatation that promote placental perfusion. Additional mechanisms could involve increased oxygen delivery to the fetus as a result of shift of the oxygen dissociation curve and increased NO bioavailability as a result of reduced scavenging by hemoglobin.

P2-057

Fetal Origin of Adiposity: Role of Maternal and Peri-natal Micronutrient Status

M. Raghunath, L. Venu, Y. Durga Kishore, and I. J. N. Padmavathi. Endocrinology and Metabolism Division , National Institute of Nutrition , Hyderabad , India – 500 007.

Background: Role of maternal macronutrient deficiency (intrauterine and early postnatal) in predisposing the offspring to insulin resistance syndrome and associated diseases including obesity has been studied earlier. We have assessed the effect of maternal micronutrient (MN) deficiencies in predisposing the offspring to obesity in their later life. **Methods:** Female, weanling, wistar/NIN rats consumed for 9-12 wks, a control diet (n = 5-7) or the same with 50% restriction of minerals (MR) or vitamins (VR)(n=15-18); or 70% restriction of magnesium (MgR) or zinc (ZnR) (n=21) and then mated with control males. A third of the restricted dams were rehabilitated from parturition and their pups from weaning . The pups from the remaining restricted dams were weaned to control diet or continued on restricted diet. Each of the groups had eight male offspring from weaning onwards. Body adiposity of the offspring was determined by measuring total body electric conductivity, plasma lipid profile and adiponectin levels on postnatal day 90 and 180. Parameters of tissue oxidative stress (levels of malondialdehyde and protein carbonyls) and antioxidant status (reduced glutathione, catalase, superoxide dismutase and glutathione peroxidase) were determined in the liver of the offspring on postntatal day 180. **Results:** MR and ZnR pups had significantly lower birth weights than controls while all the MN restriction regimens studied, decreased the weaning weight of the pups. At weaning as well as on postnatal day 90 and 180, the body mass index (BMI) of MR and ZnR offspring was lower than that of controls, while VR and MgR pups had BMI comparable to controls. MR, VR and MgR offspring had significantly higher body fat% (10 - 45%) than controls, lower lean body mass and fat free mass at three months of age but no such effect was seen in ZnR offspring. MR or MgR induced changes could at best be corrected partly by rehabilitation; whereas VR induced changes could be reversed completely by rehabilitation from parturition but not weaning. Interestingly, both the rehabilitation regimens increased body fat % of ZnR pups by 30 -34% at 90 but not 180 days of age. VR but not MR or MgR was associated with increased oxidative stress and lower circulating adiponectin levels. **Conclusions:** Maternal and peri-natal micronutrient status altered the body adiposity of the offspring, a forerunner of insulin resistance syndrome and related diseases in adulthood.

P2-058

Maternal Free Cortisol in Early Pregnancy and Fetal Sex - Effects on Maternal Cortisol Response and Fetal Heart Rate Reactivity to Acute Psychosocial Stress

Margarete I. Rieger [a], Harald Wurmser [b], Angelika Buske-Kirschbaum [c], Mechthild Papoušek [b], Karl-Martin Pirke [a], Dirk Hellhammer [a]; [a] Department for Theoretical and Clinical Psychobiology, University of Trier, Johanniterufer 15, 54290 Trier, Germany; [b] Biological Psychology Technical University of Dresden, Zellescher Weg 17, 01062 Dresden, Germany [c] Child Care Centre, Ludwig-Maximilians-University Munich, Heiglhofstraße 63, 81377 Munich, Germany

Background: A considerable body of research has documented gender differences in physiological responses to stress in adults. Animal studies show gender-dependent effects of prenatal stress. Sex differences in the prevalence of stress related diseases in humans are well documented. Several studies suggest that enhanced cortisol levels during pregnancy influence fetal brain development and produce long-term changes in offspring's behavior. These abnormalities are associated with modifications in the synthesis and release of certain neurotransmitters. Up to now, it is unclear which mechanisms cause gender-dependent effects of adverse prenatal conditions. The aim of the presented study was to investigate if maternal cortisol and fetal sex affect maternal cortisol response and fetal heart rate (FHR) reactivity to an acute social stressor (Trier Social Stress Test, TSST) and if this reactivity predicts neonatal stress reactivity and habituation in the Neonatal Behavioral Assessment Scale (NBAS). **Methods:** Seventy-four mother-fetus dyads were assessed at 32-34 gestational weeks using a cardiotocograph. Mothers were asked to collect saliva samples in early and late pregnancy to measure the rise of free cortisol after awakening. Additionally, maternal cortisol response to the TSST was assessed. Analyses were performed on the 15 min baseline, 10 min stress, and 10 min post-stress periods. Mean FHRs were computed for each phase as well the FHR increase and post-stress decrease. Neonates were assessed using the BNBAS. **Results:** Data indicate a significant increase in FHR in response to the TSST ($F=54.45$, $p<.0001$, $\omega^2=.46$). Free saliva cortisol levels in early pregnancy were associated with enhanced FHR during all three test-phases ($F=9.93$, $p<.01$, $\omega^2=.15$). FHR reactivity and recovery were related to stress reactivity in the BNBAS. Surprisingly we found an effect of fetal sex on maternal cortisol response to the TSST ($F=8.11$, $p<.01$, $\omega^2=.11$). Although we did not found any sex differences in FHR response and cortisol awakening rise, pregnant women with female fetuses showed higher cortisol levels and a pronounced cortisol response to the TSST. **Conclusions:** Results support the hypothesis that maternal HPA activity in early but not in late pregnancy influences ergotropic or autonomic development of the fetus. Results also suggest that prenatal FHR patterns may be used as predictors for neonatal behavior and habituation. The effect of fetal gender on maternal cortisol secretion is difficult to interpret. Potentially, this is one mechanism to explain results that prenatal stress on HPA function is substantially more noticeable in females than in males.

P2-059

Antenatal Betamethasone Treatment Enhances Development of Ovine Fetal Brain Function at the expense of a Persistently Altered Sleep Architecture

M. Schwab[1], K. Schwab[2], T. Groh[1], M. Kott[1] P.W. Nathanielsz[3] and O.W. Witte[1]; [1]Dept. of Neurology, [2]Institute for Medical Statistics and Computer Science, Friedrich Schiller University, Jena, Germany, [3]Center for Pregnancy and Newborn Research, Department of Obstetrics and Gynecology, University of Texas, San Antonio, TX, USA

Objective: We examined the effects of betamethasone administered to accelerate fetal lung maturation on development of fetal sleep states and electrocortical activity. **Methods:** Chronically instrumented pregnant sheep were treated with single (112 days gestation, dGA, term 150 dGA) or repetitive courses of betamethasone phosphate (Celestan solubile) at 106, 112 and 118 dGA (n=8) or an equal amount of saline (n=7). Each course consisted of 2x110µg/kg betamethasone 24h apart equivalent to 2x8 mg betamethasone administered to a 70 kg pregnant woman. Fetal electrocorticogram (ECoG) was recorded continuously between 105-130 dGA. Artifact-free one hour ECoG-epochs were analyzed using spectral analysis. **Results:** At 106 dGA, ECoG showed a predominantly premature REM sleep pattern. Administration of betamethasone at this age leads to maturation of REM sleep but not NREM sleep ECoG. The power spectrum of the ECoG reached a maturational state at 112 dGA that was similar to that in controls at 130 dGA (Fig. 1). Repetitive treatment did not have any further effect on ECoG maturation. Early maturation of REM sleep was accompanied by fragmentation of REM and NREM sleep states that remained fragmented until the end of the examination period. **Conclusions:** A single course of betamethasone induces maturation of cortical neuronal activity but not of thalamic pacemaker circuits at the expense of sleep state fragmentation. Sleep state fragmentation has been shown to continue until adulthood after prenatal stress in rats. The absence of acute suppression of the ECoG during repeated courses of betamethasone indicates programming of glucocorticoid resistance. The extent to which these changes affect postnatal brain function remains to be determined.

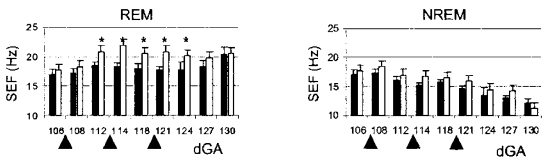


Fig. 1 Effects of three courses betamethasone (arrows) on development of spectral edge frequency (SEF) as a sensitive measure of frequency distribution in the power spectrum. Mean+SEM, controls n=7 (filled bars), betamethasone n=8 (open bars), *$p<0.05$ compared to controls. Arrows indicate betamethasone adimistration.

P2-060

Does Diarrhoea and Dehydration in Infancy Predispose to Higher Blood Pressure in Later Life?

George Davey Smith[1], Debbie A Lawlor[1], Sam Leary[2], Richard Mitchell[3], Andy Ness[2], Shah Ebrahim[1]. [1] Department of Social Medicine, University of Bristol, UK. [2]ALSPAC, Community Based Medicine [3] Research Unit In Health, Behaviour and Change, University of Edinburgh

Background: There is considerable evidence of early-life contributions to blood pressure. One possible mechanism would be through phenotypic plasticity in which the evolved ability to adapt to environmental stressors can lead to later life physiological dysregulation. Fluid loss - through severe diarrhoea and dehydration in infancy - could lead to greater sodium and fluid retention, as the ability to adapt in such a way would reduce the risk of death during subsequent episodes of diarrhoea and dehydration. However, in the long term this adaptation may result in high blood pressure. Evidence from animal studies and some limited human evidence supports this hypothesis. **Methods:** We conducted two studies. First, we related blood pressure at age 7 in the ALSPAC study to a history of hospitalisation for dehydration in the first 6 months of life. Second, we examined the association between temperature and rainfall during the first year of life and blood pressure among British women aged 60 – 79. The reasoning behind this is that in the earlier decades of the 20th century high temperature and low rainfall were associated with high rates of infancy diarrhoea and infant mortality. Therefore women who experienced high temperatures and low rainfall during their first year of life would have been at increased risk of having experienced severe infant diarrhoea. **Results:** Of the 7,840 ALSPAC cohort members with blood pressure measures at age 7, six had been admitted to hospital in the first 6 months of life for

dehydration. This group had higher systolic (1.5; -5.8, 8.9) and diastolic (5.9; 0.6,11.3) mmHg blood pressure than the whole cohort; adjustment for sex, age, height and body mass index at the time of blood pressure measurement, birthweight, gestational age, and maternal education had little influence on these findings, which if anything strengthened slightly. Among the 3,964 women aged 60-79 years of age higher temperature during the first year of life and less rainfall were both independently associated with higher systolic but not diastolic blood pressure. A standard deviation higher temperature was associated with 1.25 (0.46,2.04) mmHg higher systolic blood pressure and a standard deviation higher summer rainfall was associated with 1.72 (0.90,2.52) mmHg lower systolic blood pressure. Adjustment for a wide range of confounding factors had little influence on these findings. As expected, temperature and rainfall in the first year of life were not related to confounding factors. **Conclusions:** These two studies, while both with limitations, support the hypothesis that the experience of diarrhoea and dehydration in infancy is related to later higher blood pressure. Such a mechanism could contribute importantly to levels and trends in blood pressure within populations and could also provide evidence for possible primordial prevention of raised blood pressure. Further evidence, in particular from follow-up of randomised controlled trials of prevention of infancy diarrhoea, could provide robust evidence on this hypothesis.

P2-061

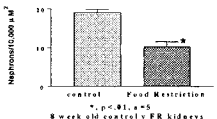
The Impact of "Size at Birth" Definition on the Relationship Between Early Exposures and Risk of Cardiovascular Disease in Polish Population of Persons Aged 24-29 Years Katarzyna Szamotulska [1], Dorota Szostak-Wegierek [2 1)] Department of Epidemiology, National Research Institute of Mother and Child, Warsaw, Poland [2)] Department of Nutrition Related Diseases, National Food and Nutrition Institute, Warsaw, Poland.

Background: Findings from studies investigating relationship between early exposures and risk of adult chronic disease vary depending on the definition of size at birth. **Aim:** To explore the impact of different "size at birth" definitions on the relationship between cardiovascular risk factors in young adults and size at birth. **Methods:** The study group consists of persons aged 24-29 years, whose mothers participated in the prospective cohort study of risk factors of low birth weight during pregnancy conducted at the National Research Institute of Mother and Child in Warsaw in 1974-77. Original structured questionnaires from this study collected at the first and second visit during pregnancy and after delivery have been stored for 1912 mother until now. All mothers were asked for help in inviting their children to physical examination and fasting blood samples testing at the National Food and Nutrition Institute in 2000-2004. 495 children agreed to participate (214 men and 281 women). Multifetal pregnancies and diabetic women were originally excluded from the study in the 70-ties. Men and women taking blood pressure, lipids or glucose lowering drugs and women taking oral contraceptives were additionally excluded from the current study. Systolic and diastolic blood pressure, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, fasting glucose, fasting insulin, HOMA-IR index and HOMA-βC index were used for the analysis of cardiovascular risk factors. Gestational age, birth weight of all infants, birth weight of infants born at 37th week of gestational age or later, ponderal index of infants born at 37th week of gestational age or later, birth length of infants born at 37th week of gestational age or later, birth weight less than 10 percentile for gestational age and quartiles of birth weight for gestational age were explored as "size at birth" variables. Linear regression analysis on transformed parameters of coronary risk factors adjusted for current body mass index was applied. **Results:** Among investigated cardiovascular risk factors, the most consistent results were seen, when size at birth was defined as birth weight less than 10 percentile for gestational age or quartiles of birth weight for gestational age. Triglycerides concentration, fasting insulin, HOMA-IR and HOMA-βC were inversely, although not statistically significantly, related to quartiles of birth weight for gestational age, in every group of actual body mass index, both in men and women. Estimated HOMA-IR in men with BMI less then 25.0 was 1.6 in the lowest quartile of birth weight and 1.2 in the highest quartile, in men with BMI 25.0-30.0 – 2.3 in the lowest quartile and 1.8 in the highest quartile, and in men with BMI more than 30.0 – 4.5 in the lowest quartile and 3.5 in the highest quartile. **Conclusions:** "Size at birth" definition in studies on the relationship between early exposures and risk of adult chronic disease is one of key issues in life course approach research.

P2-062

Nephrogenic Phenotype of Hypertensive Offspring Exposed to Maternal Undernutrition in Utero John S. Torday[1,2], Mina Desai[2], Virender K. Rehan[1], Michael G. Ross[2]. [1]Department of Pediatrics, [2]Department of Obstetrics and Gynecology, David Geffen School of Medicine at UCLA and LABioMed at Harbor-UCLA Medical Center, Torrance, CA 90502, USA

Background: Deficit in nephron endowment itself could be the renal risk factor for hypertension. Demographic groups in whom hypertension is unusually prevalent tend to have smaller kidneys, implying fewer nephrons, and some inbred hypertensive rat strains have, on average, fewer nephrons than their respective normotensive counterparts. Recent observations in humans relate low birth weight and fewer nephrons at birth to an increased risk of hypertension in later life. Moreover, experimental studies in rodents suggest that maternal protein intake during gestation is directly related to the numbers of nephrons formed, and when protein intake is restricted, the offspring develop hypertension at maturity. We have previously shown that maternal nutrient restriction results in intrauterine growth restricted newborns which are hypertensive as adults. We hypothesized that maternal food restriction causes suppression of fetal/newborn nephrogenesis and impaired renal development, contributing to offspring hypertension. **Methods:** Pregnant Sprague Dawley rats and offspring were studied. Control dams (n=5) received ad libitum food, whereas study dams (n=5) were 50% food-restricted from pregnancy days 10 to 21. At birth, litter size was culled to 4 males and 4 females. All pups were nursed by dams fed ad libitum and were weaned at 3 weeks to ad libitum feed. Kidney wet weight was measured at 1 day of age and morphometric determination of glomerular number was assessed in 8 week old control and maternally food-restricted (FR) offspring. The kidneys were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned longitudinally in 10 micron sections and stained with hematoxylin and eosin. The number of glomeruli per kidney was determined using a standard reticule. Data were analyzed by Student's t-test. **Results:** At 1 day of age, FR pups had significantly lower body weights (6.0±0.3 vs 7.1±0.3 g, $p<0.01$) and reduced kidney weights (0.65±0.01 vs 0.80±0.01 g, $p<0.001$) as compared to the controls. However, when kidney weights were expressed relative to body weights, no significant differences were evident. Notably, at 8 weeks of age, there were 46% fewer glomeruli per kidney in the FR offspring vs controls (Figure).



8 week old control v FR kidneys

Conclusion: Nutritional status in utero appears to have a major impact on renal structure and development, particularly, nephron number. Reduced nephrogenesis may potentially be the mechanism involved in development of hypertension in intrauterine growth retarded adults.

P2-063

Antenatal Dexamethasone Treatment Induces Accelerated Aging in the Adult Mouse Cornelle W Noorlander, Gerard HA Visser, Peter GJ Nikkels, Pierre NE de Graan. Rudolf Magnus Institute of Neuroscience, Department of Obstetrics and Pathology, University Medical Center Utrecht, The Netherlands.

Background. Synthetic glucocorticoids (GCs) are frequently administered to pregnant women at risk for preterm delivery. Follow-up of infants exposed prenatally is thus far reassuring. However, long term follow-up is lacking. To address this issue, we treated pregnant mice with either a therapeutic dose of dexamethasone (DEX) or saline at embryonic day 15.5 and studied hippocampal development, behaviour at the age of six months and histology of heart and liver. **Results.** Following DEX there was an increased apoptosis in the dentate gyrus. This was followed by an increased cell proliferation, but at adult age cell proliferation was decreased. In addition, electrophysiological experiments showed a small induction of long term depression (LTD) in hippocampal slices. mRNA expression of NMDA receptor subunit NR2B, which is involved in LTD, was decreased. Spatial learning in reference and working memory of the Morris Maze was decreased in DEX treated mice. There was moderate central fibrosis in the adult liver and interstitial fibrosis in the adult heart after DEX administration. **Conclusions.** Antenatal DEX treatment in mice results in long term molecular changes, cognitive deficits, impaired synaptic plasticity and in histological changes in liver and heart, all indicating accelerated aging.

P2-064

Maternal Haemodynamics During Pregnancy Predict Offspring Haemodynamics During Childhood Marilyn B. Lawrence Wright, Michael S. Boyne, Clive Osmond, Tamika Y.N. Royal, Terrence E. Forrester; Tropical Medicine Research Institute, The University of the West Indies, Kingston, Jamaica and Medical Research Council Environmental Epidemiology Unit, University of Southampton, England

Background: Rate pressure product, calculated as blood pressure multiplied by the heart rate is related to the long term load on the left ventricle (and thus myocardial oxygen demand) and the systemic arteries. We hypothesized that maternal rate pressure product during pregnancy influences the blood pressure of the offspring. **Methods:** The 486 mother-offspring pairs analysis were members of the Vulnerable Window Cohort, followed longitudinally at University of the West Indies, Kingston, Jamaica since 1993. Maternal haemodynamic, anthropometric, biochemical and haematological status was assessed throughout the pregnancy. Foetal and placental growth were assessed by maternal abdominal ultrasound and birth anthropometric measurements were made using standardized techniques. Mothers and children have been seen twice a year since then for anthropometric, blood pressure and pulse rate measurements. Multivariate regression models were created using STATA 8.0. **Results:** Mother's systolic blood pressure during pregnancy ($\beta = 0.187$, $p < 0.001$), child's current weight ($\beta = 0.342$, $p < 0.001$) and having a birthweight in the highest quartile ($\beta = -0.183$, $p < 0.05$), were predictive of child's systolic blood pressure. Mother's pulse rate during pregnancy, the child's current weight, the child's gender and birth weight were predictive of child's pulse rate. (p-values < 0.05). Mother's pulse pressure during pregnancy ($\beta = 0.134$, $p < 0.001$), child's current weight ($\beta = 0.204$, $p < 0.001$) and having a birthweight in either the second ($\beta = -0.165$, $p < 0.05$), third ($\beta = -0.165$, $p < 0.05$) or highest quartile ($\beta = -0.263$, $p = 0.001$), were predictive of child's pulse pressure. Mother's rate pulse pressure product ($\beta = 0.125$, $p < 0.001$), child's current weight ($\beta = 0.173$, $p < 0.001$), child's gender ($\beta = 0.131$, $p < 0.05$) and having a birth weight in the third ($\beta = -0.242$, $p = 0.001$) or highest quartile ($\beta = -0.308$, $p < 0.001$), were predictive of child's rate pulse pressure product. Mother's rate systolic pressure product ($\beta = 0.145$, $p < 0.001$), child's current weight ($\beta = 0.195$, $p < 0.001$), child's gender ($\beta = 0.135$, $p < 0.05$) and having a birth weight in the third ($\beta = -$

0.186, p < 0.05) or highest quartile (ß = -0.228, p < 0.01), were predictive of child's rate systolic pressure product. **Conclusion:** Offspring haemodynamics during childhood are predicted not only by current childhood gender and weight, but also by child birthweight and even more strongly and more consistently, by maternal haemodynamics during pregnancy.

P2-065

Haemodynamics During Childhood are Predicted by Foetal Growth and Development Marilyn B. Lawrence Wright, Michael S. Boyne, Clive Osmond, Tamika Y.N. Royal, Terrence E. Forrester; Tropical Medicine Research Institute, The University of the West Indies Kingston, Jamaica and Medical Research Council Environmental Epidemiology Unit, University of Southampton, England

Background: Rate pressure product, calculated as blood pressure multiplied by heart rate is related to the long term load on the left ventricle and the systemic arteries and is correlated with myocardial oxygen demand. We therefore sought to determine whether there is developmental programming of one's rate pressure product. **Methods and Results:** The growth, development and haemodynamics of 486 childhood participants in a Jamaican cohort of children who have been followed longitudinally since 1993. Foetal and placental growth were assessed by maternal abdominal ultrasound at 14, 17, 20, 25, 30 and 35 weeks gestation. At birth, placental weight as well as child weight, length, head, chest, mid upper arm and abdominal circumferences were measured using standardized techniques. The children have been seen twice a year since then for anthropometric, blood pressure and pulse rate measurements.

Variable	Regression coefficient	Standard Error	p-value	95% Confidence Interval
Birth weight (kg)	-0.113	0.027	0.000	-0.165, -0.060
Head circumference (cm)	-0.055	0.018	0.002	-0.090, -0.021
Abdominal circumference umbilicus (cm)	-0.039	0.012	0.001	-0.062, -0.016
Crown heel length (cm)	-0.029	0.009	0.002	-0.047, -0.010
Crown rump (cm)	-0.043	0.012	0.000	-0.067, 0.020
Placental weight (g)	-0.001	0.000	0.001	-0.001, -0.000
Gestational age (days)	-0.008	0.002	0.000	-0.012, -0.004
Mid-upper arm circumference (cm)	-0.065	0.027	0.017	-0.117, -0.012
Chest circumference (cm)	-0.050	0.013	0.000	-0.075, -0.025
Placental volume @14 weeks (mm)	-0.097	0.030	0.001	-0.156, -0.039
Placental volume @17 weeks (mm)	-0.091	0.028	0.001	-0.146, -0.036
Placental volume @20 weeks (mm)	-0.066	0.029	0.025	-0.123, -0.008
Femoral length @ 35 weeks (mm)	-0.068	0.030	0.025	-0.127, -0.009

Conclusion: A higher rate pressure product and thus increased myocardial oxygen demand during childhood is associated with smaller foetal and placental size as measured in utero and at birth.

Diabetes / Metabolic Syndrome

P2-066

Insulin Resistance in the Non-oxidative Glucose Metabolic Pathway in Young Men who had Low Birth Weight Julie Solberg Appel[1,2], Charlotte Brøns[1,2], Louise Grunnet[1], Christine Bjørn Jensen[1], Heidi Storgaard[1], Arne Astrup[2] and Allan Vaag[1] [1]Steno Diabetes Center, Gentofte, Denmark; [2]The Royal Veterinary and Agricultural University, Frederiksberg, Denmark.

Background & Aim. Muscle insulin resistance is an early defect in humans in whom Type 2 Diabetes (T2D) later develop, and a consistent early finding in subjects with low birth weight (LBW), supporting the idea of a fetal origin of insulin resistance and T2D. Using the gold standard euglycaemic clamp technique, we recently reported normal whole-body insulin action and non-oxidative glucose metabolism (NOGM) in the face of a minor impairment of insulin-stimulated glycolysis in lean Caucasian 19-yr old men who had LBW. In order to further elucidate the early course of metabolic events leading to whole-body insulin resistance in LBW subjects, we studied slightly older men with LBW (24 yrs), using an optimized clamp procedure including higher physiological plasma insulin levels. **Protocol.** Fifty-eight 22-24-yr old male volunteers were recruited from the Danish National Birth Registry (LBW): N=27, BW=10th percentile; normal birth weight (NBW): N=31, 50th=BW=90th percentile). All were born at term, and subjects with family history of diabetes or BMI = 30 were excluded. The subjects underwent an intravenous glucose tolerance test (IVGTT) followed by a three hour 80mU/m^2/min hyperinsulinemic euglycemic clamp in combination with indirect calorimetry. Estimates of glucose uptake (M-value), Si (M-value/ins$_{clamp}$), oxidative and non-oxidative glucose metabolism (OGM, NOGM), lipid oxidation (LIPOX), as well as first phase insulin response (FPIR) and Di (FPIR x Si) were obtained. Body fat was determined by DXA scan. All data are means (± SD). **Results.** LBW subjects had significantly elevated fasting blood glucose (4.8±0.53 vs. 4.5±0.42 mM, *P*=0.016) and insulin levels (41.4±15.6 vs. 30.7±14.1 pmol/L, *P*=0.003) as compared to NBW subjects. In addition, LBW subjects were more abdominally obese (trunk fat mass (g) / total fat mass (g): 0.53 ± 0.04 vs. 0.50 ± 0.05, *P*=0.02; Trunk fat mass (%) / leg fat mass (%):1.23 ± 0.17 vs. 1.08 ± 0.2, *P*=0.02, BMI (24.6 ± 3.5 vs. 23.2 ± 2.8, *P*=0.10). Glucose uptake was slightly but significantly reduced in LBW (M-value: 12.52 ± 3.08 vs. 14.20 ± 2.94 mg/FFM/min, *P*=0.02). Interestingly, insulin resistance was explained solely by a reduction in NOGM (8.12 ± 2.79 vs. 9.81 ± 2.77 mg/FFM/min, *P*=0.03), while basal and insulin stimulated rates of glucose oxidation were normal. A modest non-significant increase in FPIR in LBW (2309±1551 vs. 1884 ± 1320, *P*=0.09) matched the reduction of M-value, and hence no change in Di was observed. **Conclusion.** We report that at the early stages, when whole-body insulin resistance first occurs, the predominant metabolic defect in LBW subjects is in non-oxidative glucose metabolism. Importantly, impaired non-oxidative glucose metabolism and muscle glycogen synthesis is the major defect explaining insulin resistance in first degree relatives of type 2 diabetic patients, further supporting the role and relevance of fetal programming in insulin resistance and T2D. Our data suggest that the observed metabolic changes in LBW subjects may to some extent be secondary to an increased abdominal fat mass.

P2-067

Insulin and Insulin Sensitivity are Major Predictors of Maternal Weight Change and Estimated Birth Weight in Normal Pregnancies Ueland T*[#], Dalsoren T*, Voldner N[§], Godang* K, Henriksen T[§], Bollerslev J*. Section of Endocrinology*, Research Institute for Internal Medicine[#] and Department of Obstetrics and Gynecology[§], Rikshospitalet University Hospital and University of Oslo, Rikshospitalet, Oslo, Norway.

Background*:* The prevalence of macrosomic children has gradually increased during the last decade, and is associated with short and long term maternal and fetal complications. Fetal growth is determined by different factors, including genes, environment, nutrition, hormones and maternal anthropometric parameters. **Aim***:* We investigated maternal plasma parameters and indices of glucose intolerance at different time points in the course of normal pregnancy as independent predictors of maternal weight gain and if these are related to estimated birth weight of the offspring. **Methods***:* Blood samples during oral glucose tolerance test and maternal and fetal anthropometric measures were collected from 44 normal pregnant women at week 14-16 and 30-32 weeks of gestation. Birth weight was estimated at 40 week. The relationship between maternal weight gain/estimated birth weight and potential predictors was analyzed by univariate analysis followed by stepwise linear regression. **Results***:* Spontaneous insulin secretion and gender were significant predictors explaining 40% of the variation in estimated birth weight, while the maternal weight gain was best explained by changes in insulin sensitivity and parameters reflecting increased fat mass. *Conclusion:* Gender, maternal fasting insulin and insulin sensitivity were significant determinants of estimated birth weight in normal pregnancies without gestational diabetes. The finding that weight gain was not a predictor of birth weight, despite being associated with insulin sensitivity may be related to fact that women who weighed less at baseline are those who increased most in weight and develop poorer insulin sensitivity during the course of pregnancy.

P2-068

Gonadotrophin Stimulation in IVF Cycles Worsens Insulin Resistance in Women with Polycystic Ovary Disease Chantal D. Simonis, Christopher D. Byrne, Malcolm C. Richardson, Sarah H. Wild* and Iain T. Cameron. Developmental Origins of Health and Disease, School of Medicine, University of Southampton, Southampton, UK and *Public Health Sciences, University of Edinburgh, Edinburgh, UK.

Background. Although there is a strong association between circulating androgen concentrations and insulin resistance in polycystic ovary syndrome (PCOS), the identity of the primary event is unknown. To investigate the possibility that increased androgens are causal in the deterioration of insulin resistance, we measured these parameters in individuals with PCOS undergoing stimulation with gonadotrophin for IVF. **Methods.** A control group of 14 endocrine-normal women, and an experimental group of 9 women with PCOS were recruited from an IVF programme. PCOS was diagnosed by the presence of 2 out of 3 of the following criteria: 1) oligomenorrhoea, 2) clinical and/or clinical signs of hyperandrogenism, 3) polycystic ovaries viewed on ultrasound. Baseline fasting measurements were undertaken before recombinant follicle stimulating hormone (FSH) treatment, and repeated on the day of oocyte retrieval, at maximal FSH stimulation. Fasting concentrations of glucose, insulin, estradiol, testosterone, androstenedione and sex-hormone binding globulin were measured. Insulin resistance (IR) was estimated using 'homeostatic model assessment' (HOMA). **Results**. Pre- and post-treatment median values for the two groups are summarised in the following table:

	Pre-treatment		Post-treatment	
	control	PCOS	Control	PCOS
Androstenedione (nmol/l)	8.7	14.6	10.7	32.3
Testosterone (nmol/l)	1.25	2.2	2.4	5.6
HOMA	2.09	2.46	1.1	3.4

Androgen concentrations increased after FSH treatment in both groups (P<0.001, post-treatment androstenedione and testosterone vs baseline in both groups). Higher values for androgens in the PCOS group in the baseline study, were also evident post-treatment (testosterone, 2.3-fold vs control; androstenedione, 3.0-fold vs control) although at much higher circulating values. There was a rise in insulin resistance after treatment only in the PCOS group (P<0.02). The presence of PCOS, and post-treatment androstenedione and testosterone concentrations were associated with post-treatment HOMA (P<0.003, P=0.002, P=0.001 respectively). The presence of PCOS and post-treatment androgen concentrations accounted for 48% of the variance in post-treatment HOMA (R^2=0.48, P=0.005). **Conclusion.** We conclude that in PCOS, gonadotrophin stimulation raises circulating androgens to a threshold value where there appears to be

an impact on insulin resistance. We speculate that the adverse change in insulin resistance in PCOS may have implications for oocyte quality and development of the early embryo in these patients following gonadotrophin therapy.

P2-069

Evidence of a Critical Birthweight for Increased Risk of the Metabolic Syndrome in African Children Noël Cameron*, Melanie M Wright*, Paula L Griffiths*, Shane A. Norris† and John M. Pettifor†; *Centre for Human Development and Aging, Department of Human Sciences, Loughborough University, Loughborough LE11 3TU, UK and †Mineral Metabolism Research Unit, University of Witwatersrand, Johannesburg, South Africa

Background Adipose rebound (AR) is the age at which BMI increases after its nadir in childhood, typically occurring at six years. An earlier AR is associated with a higher BMI at AR and an increased risk of obesity later in life. Catch-up growth during infancy is associated with a higher BMI during childhood and thus such children might be expected to also have an earlier AR and a higher BMI at AR compared to their peers. **Methods** Participants were 154 African children (boys = 87) from the Birth to Twenty birth cohort study based in Johannesburg/Soweto, South Africa. The parametric Reed model (1) was applied to mixed longitudinal height and weight data to derive values at whole-year time-points from birth up to 10 years of age. Subsequent patterns of BMI were produced from which age at AR was derived from fitted polynomials. Rapid growth was defined as an increment of > 0.67 weight-for-age Z scores from birth to two years. Inclusion criteria were: normal gestation (37 to 41 weeks), minimum of 5 height and 6 weight assessments (including birth weight and data at two years) and assessment of prepubertal status at final examination. **Results** The mean (SD) age at AR were 5.7 (1.2) and 6.0 (1.2) years (P=0.197) and BMI at AR were 15.3 (1.2) and 14.7 (1.0) kg/m^2 (P=0.004) for boys and girls respectively. Significant predictors of BMI at AR were identified as age at AR, the presence or absence of rapid growth (RG), birthweight Z-score (BWAZ) and the interaction between RG and BWAZ: BMI at AR =16.008+(-0.184*Age at AR)+(1.584*RG)+(0.252*BWAZ)+ (0.730*RG*BWAZ) SEE = 1.07. RG = 1 for a child showing rapid growth and 0 for remaining children. Age at AR, RG, BWAZ and the RG*BWAZ interaction were statistically significant at P<0.012. RG*BWAZ produced a threshold BWAZ of –2.17 above or below which RG children had a greater or lesser BMI at AR compared to their peers. This threshold is equivalent to a birthweight of 2380g for boys and 2160g for girls. **Conclusions** Age at AR was negatively associated, and the presence of RG during infancy, BWAZ, and the RG *BWAZ interaction were positively associated with BMI at AR. RG boys and girls born above 2380g and 2160g respectively, had a higher BMI and an earlier age at AR and thus an increased potential to be obese and suffer from metabolic syndrome in adolescence or adulthood, compared to their peers. Conversely, RG infants possessing a birthweight below those critical values had a lower potential to develop risk factors for obesity or the metabolic syndrome. Reference 1.Berkey CS, Reed RB. A model for describing normal and abnormal growth in early childhood. Hum Biol 1987;59:973-87.

P2-070

Maternal Pregravid Body Mass Index (BMI) is a Predictor of Childhood Obesity in Offspring of Women with Normal Glucose Tolerance (NGT) and Gestational Diabetes (GDM). Patrick Catalano, Kristen Farrell, Larraine Presley, Saeid Amini. Dept. of Reproductive Biology, Case Western Reserve University @ MetroHealth Medical Center, Cleveland, Ohio 44109 USA.

Background. There has been a significant increase (mean 116 g) in term singleton birth weight in our population in the past 30 years (n = 78,500). Elevated maternal BMI had the strongest relationship with the increase in birth weight (R^2 =0.08, p= <0.0001). In a body composition study of 415 infants of women with NGT and GDM, maternal pregravid BMI has the strongest relationship with neonatal fat mass (R^2 =0.066, p=0.0001) and % body fat at birth (R^2 =0.072, p=0.0001). The purpose of this prospective cohort analysis was to examine the affect of maternal pregravid obesity (BMI) as a predictor for obesity in offspring of NGT and GDM in childhood. **Methods.** Ninety infants (53 NGT, 37 GDM), were prospectively evaluated at birth and again at 6 to 11 years of age. Thirty-nine males and 51 females were evaluated. At birth estimates of body composition were performed using anthropometrics and total body electrical conductivity (TOBEC). At follow-up (mean ± SD) of 8.9 ± 1.8 years, weight for all subjects was classified based on CDC criteria (age and gender specific); body composition was measured using Dexa on 63 children. Additional factors in the models included paternal height and weight, maternal age, parity, weight gain and smoking history during the index pregnancy. Birth information such as gestational age, Z score, ponderal index, and breast or bottle feeding was included in the model. At follow-up, models were generated to predict the upper quartile for CDC weight criteria and upper tertile for body fat mass. **Results.** Maternal pregravid BMI was the strongest predictor of classification into the upper quartile of the CDC criteria for weight and gender. (p=0.0096, OR 3.86, 95% CI 1.4-10.7). When the model was adjusted for % body fat at birth this relationship was improved (p=0.008, OR 4.08, 95% CI 1.4-11.8). Furthermore when adjusted for % body fat at birth, gestational age at delivery, group (GDM vs. NGT), maternal weight gain in pregnancy and parity; maternal pregravid BMI remained a significant (p=0.04) predictor of a child's classification into the upper CDC quartile for weight. Maternal pregravid BMI was also the strongest predictor of classification of a child being in the highest tertile of fat mass at follow-up (p=0.001, OR 7.79, 95% CI 2.25, 26.9) after adjusting for gender. Pregravid BMI remained a significant (p=0.02) predictor of fat mass in childhood, after adjusting for both gender, and the mother's GDM status during pregnancy. **Conclusions.** Maternal pregravid BMI is a significant predictor for increased adiposity both at birth and at the time of 6-11 year follow-up. Supported by NIH grant:HD22965 and General Clinical Research Center Grant RR-80.

P2-071

Long-Term Follow-up of Infants of Women with Normal Glucose Tolerance (NGT) and Gestational Diabetes (GDM), Risk Factors for Increased % Body Fat and Components of the Metabolic Syndrome in Childhood. Patrick Catalano, Kristen Farrell, Larraine Presley, Saeid Amini. Dept. of Reproductive Biology, Case Western Reserve University @ MetroHealth Medical Center, Cleveland, Ohio 44109 USA.

Background. The purpose of this prospective longitudinal cohort analysis was to determine the maternal and perinatal factors related to obesity and related sequela in the offspring of NGT and GDM. **Methods.** Sixty-three infants (39 NGT, 24 GDM), were evaluated at birth and at 6 to 11 years of age (28 males, 35 females). At birth estimates of body composition were performed using total body electrical conductivity (TOBEC). At follow-up (mean ± SD) of 9.6 ± 1.6 years, body composition was measured using Dexa and fat distribution using skinfolds. Additional perinatal data included maternal age, height, weight, parity, weight gain and smoking history during pregnancy as well as paternal height and weight. At birth information on gestational age, weight, Z score, body composition, and breast or bottle feeding were recorded. At follow-up, we obtained blood pressure, dietary history (3 day dietary logs), fasting glucose and insulin (HOMA estimates of insulin resistance) and lipid profile. **Results.** At follow-up data were analyzed based on tertiles of % body fat (Table). The mean percent body fat in the first tertile (T1) was 19.3%, in the second tertile (T2) was 27.5% and in the third tertile (T3) was 39.4%. Significant differences among tertiles are presented. (n=63 unless otherwise indicated). **Conclusion**. Children in T3 are more insulin resistant, have central obesity, elevated systolic blood pressure and abnormal lipid profile. Increased maternal pregravid BMI and GDM during pregnancy are significant risk factors for obesity in childhood. Supported by NIH grant: HD22965 and General Clinical Research Center grant RR-80.

P2-072

C-Reactive Protein and Metabolic Syndrome in Adult Offspring of Intrauterine Growth Restricted (IUGR) Newborns Mina Desai, Omid Khorram, Dave Gayle, and Michael G. Ross Department of Obstetrics and Gynecology, David Geffen School of Medicine at UCLA and LABioMed at Harbor-UCLA Medical Center, Torrance, CA 90502, USA

Background: C-reactive protein (CRP) is an acute-phase protein produced by hepatocytes in response to tissue injury, infection, and inflammation. An association between plasma high-sensitivity CRP concentrations and cardiovascular disease has been noted in both men and women, with strong evidence that elevated plasma CRP concentrations predict coronary heart disease. Additional studies have demonstrated an association of body fat and CRP, with increased levels in obese and/or hyperinsulinemic individuals. We have developed a rat model of gestationally programmed metabolic syndrome: Maternal food restriction results in intrauterine growth restricted (IUGR) pups which exhibit increased body fat, elevated blood glucose and plasma triglyceride levels and insulin resistance at 9 months of age. We sought to determine whether plasma CRP levels were elevated in the IUGR adult offspring and examine the association between plasma CRP levels and indices of metabolic syndrome. **Methods:** Pregnant Sprague Dawley rats and offspring were studied. Control dams received ad libitum (n=6) food, whereas study dams were 50% food-restricted (n=6) from pregnancy day 10 to 21 to produce IUGR newborns. At birth, litter size was culled to 4 males and 4 females. All pups were nursed by dams fed ad libitum and were weaned at 3 weeks to ad libitum feed. At 9 months of age, percentage body fat (DEXA), and fasting blood glucose, plasma CRP, triglycerides and insulin levels were determined. Regression analysis was used to determine association between CRP and indices of metabolic syndrome. Values (means±SEM) are presented for male offspring. **Results:** At 9 months of age, IUGR offspring were markedly heavier (742±15 vs 647±18 g, p<0.001) with greater percentage body fat (20.3±1.6 vs 12.4±1.5 %, p<0.001) and elevated blood glucose (115±2 vs 104±2 mg/dl), plasma triglycerides (97±8 vs 52±6 mg/dl) and insulin levels (0.83±0.08 vs 0.62±0.05 ng/ml). Plasma CRP levels were significantly increased in IUGR offspring (267±20 vs 190±11 ng/ml, p<0.01) as compared to controls. There was a statistically significant correlation between CRP and each component of the metabolic syndrome, including percentage body fat (r^2=0.6032, p<0.01), fasting triglycerides (r^2=0.4304, p<0.05), and insulin (r^2=0.6904, p<0.01). **Conclusion:** CRP concentrations are elevated in adult rats of nutrient restricted dams, and are highly correlated with cardiovascular risk factors. These results suggest that CRP-mediated inflammatory responses may be a contributor and/or sequelae of programmed metabolic syndrome.

P2-073

Prenatal Dexamethasone in Rats Programmes Increased Sensitivity to Hepatic Lipid Accumulation but not Obesity on a High Fat Diet Amanda J Drake, Peter J Raubenheimer, Jonathan R Seckl, Brian R Walker; Department of Child Life and Health, University of Edinburgh, Edinburgh, EH9 1UW, Endocrinology Unit, University of Edinburgh, Centre for Cardiovascular Science, Queen's Medical Research Institute, Edinburgh, EH16 4TJ, UK.

Background Low birth weight is associated with a number of cardiovascular risk factors including type 2 diabetes and the metabolic syndrome. Adult obesity modifies

this risk, although the mechanisms remain unclear. We have developed a model of programming in rats, in which in which prenatal dexamethasone exposure is associated with low birthweight and glucose intolerance in adulthood. We have used this model to explore whether dexamethasone-programming is associated with increased susceptibility to obesity and hyperinsulinaemia in animals exposed to a high fat diet. **Methods** 16 Wistar rats were treated with dexamethasone (Dex; 100 mcg/kg/day) or vehicle (Veh) subcutaneously, from day 15 of pregnancy. At birth, litters (Dex and Veh) were weighed and culled to 8. At 21 days, males from each litter were weighed and weaned on to high-fat (HF; 45% kcal from fat) or control (10% fat) diets. At 6 months, glucose tolerance tests were performed (8-10 per group), and animals were culled (n=7-9 per group). Liver triglyceride was extracted with isopropanol and measured with colorometric assay. **Results** Birth weight was reduced in Dex animals (Dex 5.34 ± 0.04g vs. Veh 6.01 ± 0.07g; p<0.001); both Dex-HF and Veh-HF were heavier than controls by 5 weeks. 6 months of HF induced obesity, hyperinsulinaemia and increased hepatic triglyceride content in both Dex and Veh groups. There were no differences between Dex-HF and Veh-HF animals in body or fat-pad weights, however Dex-HF animals had increased hepatic triglyceride content compared with Veh-HF animals (142 ± 18.9 vs. 92.2 ± 10.7 μmol/g liver, p<0.05). **Conclusions** Antenatal glucocorticoid overexposure in rats does not confer increased sensitivity to high-fat diet induced generalised obesity, but increases susceptibility to liver fat accumulation. Fatty liver is in itself a potentially serious condition which may lead to cirrhosis, but in addition, is strongly associated with insulin resistance, providing further proof that this model of low birth weight predisposes to the development of metabolic syndrome. Obesity in human and animal models is associated with altered glucocorticoid metabolism, and prenatal glucocorticoid overexposure may program enhanced glucocorticoid mediated responses. This may be a basis by which dietary obesity amplifies cardiovascular risk factors in those with low birth weight.

P2-074

Metabolic Syndrome and Related Insulin Levels in Iranian Obese Children

Pantea Ebrahimpour, Hossein Fakhrzadeh, Rasoul Pourebrahim, Anahita Hamidi, Ramin Heshmat, Mohammad Jafar Mahmoodi, Bagher Larijani;Endocrinology & *Metabolism Research Center, Doctor Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran*

Background: Insulin resistance syndrome is a cluster of metabolic abnormalities which is accompanied with an increased risk of diabetes and cardiovascular diseases. This has become an important problem in urban children due to their increasing levels of obesity. **Methods:** 535 obese 7-11years students of all the primary schools of the 6th district of Tehran were screened according to their waist circumference and then confirmed according to International obesity Task-Force (IOTF) criteria. Waist circumference, fasting serum triglycerides, high density lipoprotein-cholesterol (HDL), blood pressure, fasting plasma glucose and insulin levels were measured. **Results:** The crude prevalence rate of metabolic syndrome in these children was 20.6%. There was no significant difference between genders. The most common metabolic abnormality was hypertriglyceridemia and the less common one was low HDL levels. All the components of the metabolic syndrome were significantly more common in obese children with metabolic syndrome, except HDL levels. Moreover, insulin levels were significantly higher in these children. **Conclusion:** The prevalence of metabolic syndrome is high in Iranian obese children. Early intervention in this population who will become our future obese adults is needed not only to increase their life quality but also to decrease the future burden of diabetes and cardiovascular diseases on the society.

P2-075

Perinatal And Childhood Origins Of The Metabolic Syndrome

Rae-Chi Huang MD [1 2], Lawrence J Beilin MD [1], Valerie Burke MD [1], John P Newnham MD [3 4], Garth E Kendall PhD [5], Louis I Landau MD [6], Wendy H Oddy PhD [5 7], Kevin Blake[1], Lyle J Palmer PhD [2], Fiona J Stanley MD [5] [1]The University of Western Australia (UWA), School of Medicine and Pharmacology, Royal Perth Hospital; [2]Laboratory for Genetic Epidemiology, WAIMR, UWA, Centre for Medical Research,UWA; [3] Women and Infants Research Foundation, King Edward Memorial Hospital, Subiaco; [4]The School of Women's and Infants' Health, UWA; Telethon Institute for Child Health Research, UWA; [6]School of Pediatrics and Child Health, UWA; [7]Curtin University of Technology School of Public Health

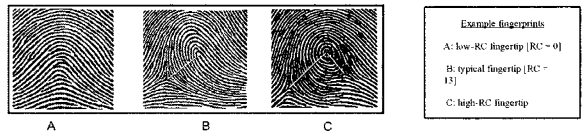
Background: The adult metabolic syndrome is a major risk factor for cardiovascular disease and will increase in prevalence with increasing childhood obesity. We sought to identify the major early life influences on the development of features of the "childhood metabolic syndrome". **Methods and Results:** Using cluster analysis on a subset (n=406) of an extensively characterized longitudinal birth cohort of Australian children, a quarter of 8 year olds had higher body mass index, serum lipids and blood pressures (BP) and a trend to higher serum glucose resembling the adult metabolic syndrome. They had a U-shaped relationship between percentage expected birth weight (PEBW) and the "childhood metabolic syndrome", and elevated BP and weight as early as 1 and 3 years of age. Increased risk of the "childhood metabolic syndrome" occurred with increased postnatal weight gain from 1-8 years old (OR=1.4, 95% CI = 1.3-1.5 per kilogram) and if mothers smoked during pregnancy (OR=1.82, CI=1.05 to 3.2). Risk was lower if children were breast fed for =4 months (OR= 0.6, 95%CI 0.37 to 0.97). Newborns in the upper two quintiles for PEBW born to smoking mothers were at increased risk of "childhood metabolic syndrome" (OR=14.0, 95% CI 3.8 to 51.1) when compared to the nadir PEBW quintile of non smokers. **Conclusion:** A U-shaped relationship for birthweight-metabolic syndrome was confirmed in a contemporary, well-nourished Western population of full term newborns. The critical difference between this and previous cohorts was the greater prominence of higher birthweights in those at risk of the "childhood metabolic syndrome". Understanding that risk is altered by early environmental factors could inform effective prevention with greatest benefits arising from reducing excess childhood weight gain and targeting the heaviest, as well as lightest newborns, particularly with a history of maternal smoking during pregnancy.

P2-076

A Fingerprint Ridge-count Gradient is Associated with Diabetes Among Middle-aged Adults: The Dutch Famine Study

Henry S. Kahn, Mariaelisa Graff, Aryeh D. Stein, L.H. Lumey; National Center for Chronic Disease Prevention and Health Promotion (CDC), *Atlanta, GA 30341 USA;* Rollins School of Public Health, Emory University, Atlanta, GA; Mailman School of Public Health, Columbia University, New York, NY.

Background: Although adult diabetes has been associated with low birth weight in some studies, the programming of diabetes may begin before the final trimester when most fetal weight gain occurs. Each fingertip's dermatoglyphic ridge count (RC) is established prior to the 20th week of gestation; thus fingerprints can be influenced only by circumstances preceding mid-pregnancy. We hypothesized that a gradient of RCs (the difference between RCs on the 1st and 5th fingertips [d15]), presumably reflecting cephalo-caudal (upper-lower) growth stimuli in early pregnancy, would be associated with the diagnosis of diabetes in middle age.



Methods: In a study of adults born around the Dutch Famine of 1944-45, 569 persons (ages 56.5 to 60.5 years) provided left- and right-hand fingerprints (ink and paper method) from which the mean d15 (Md15) could be calculated. Diabetes was identified in 82 participants by history or by a standard 2-hour glucose tolerance test, and 134 additional participants were determined to have impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). **Results:** The average Md15 was 6.8 (SD 7.6). Compared to the 353 normoglycemic persons, those with diabetes had a higher average Md15 (8.3 [SE 0.8] *vs* 6.4 [SE 0.4]; p=0.036); those with IFG/IGT had an intermediate average Md15 of 6.8 [SE 0.7]. Diabetes was not significantly associated with the RCs either on finger 1 (? = + 0.7) or finger 5 (? = -1.1). The odds ratio for diabetes (*vs* normoglycemia) for each increment of 1 SD in Md15 was 1.31 (95% CI 1.02–1.70). Adjustment for age and sex reduced the odds ratio for diabetes per SD of Md15 to 1.25 (95% CI 0.97–1.61; p=0.088). Odds ratios were unchanged by adjustments for smoking, birth weight, and several anthropometric indices tested individually (body mass index, leg-to-trunk ratio, waist-to-thigh ratio, sagittal abdominal diameter/thigh circumference, or digit-length ratio [2D:4D]). **Conclusions:** In this cross-sectional sample of Dutch adults diabetes was associated with an increased RC gradient between fingers 1 and 5 (Md15). Survivorship bias is an unlikely explanation since the participants came from a narrow range of ages only slightly older than the typical age of diabetes onset. Our findings suggest that some element of diabetes risk is associated with a marker that is itself already established in early gestation. We found little evidence that the link between early pregnancy and adult diabetes is mediated by birth weight or anthropometric variation in later life. The contribution of genes is unknown, but we recently found that Md15 is associated with aspects of the gestational environment such as season of conception and war-related dietary change. Future research into the early determinants of pancreatic beta-cell function and glucose homeostasis may benefit from using fingerprint clues to early gestational circumstances. [The findings and conclusions in this report have not been formally disseminated by the CDC and should not be construed to represent any agency determination or policy.]

P2-077

Validity of Diagnoses Related to Gestational Diabetes Based on Information from a Mandatory Hospital Discharge Registry and Telephone Interviews of Women: A Review of 3039 Patient Charts

Åse K Klemmensen[1, 2], Sjurdur F Olsen[2], Marie Louise Østerdal[2] and Ann Tabor[1,3] From the Department of [1]Obstetrics and Gynecology, H:S Hvidovre Hospital, University of Copenhagen, [2]the Maternal Nutrition Group, Danish Epidemiology Science Centre, Statens Serum Institut and [3]the Ultrasound Clinic, Juliane Marie Center, H:S Rigshospitalet

Background: Gestational diabetes mellitus (GDM) is a disease known to complicate pregnancy, cause perinatal morbidity and increase risk of diabetes and related complications later in life. We examined the validity of the GDM diagnosis when based on information from a mandatory hospital discharge registry (The National Patient Registry), with or without additional information from a structured telephone interview of the women themselves. Women participating in the Danish National Birth Cohort gave consent to further exploration in their medical history in hospital files and the National Patient registry. **Methods**: Among more than 100,000 women participating in the prospective Danish National Birth Cohort, 3084 (2.6%) gave birth at three hospitals during 1998-2000. Information regarding GDM was available from the patient registry for all women, whereas 76.6% of the women had answered the post

partum interview including questions specifically on GDM. The GMD diagnosis in the registry was in accordance with the IDC10 and represents DO244 {A-E} (gestational diabetes mellitus) and DO249 {A-C} (pregnancy with non-specified diabetes mellitus). The files of 3039 (98.5%) women were available and reviewed using a gold standard (2-hour oral glucose tolerance test) for gestational diabetes. **Results**: Sensitivity, specificity and predictive value of positive test were 58.33% (14/24), 99.64% (2204/2212) and 63.64% (14/22) for the patient registry, 50.00% (12/24), 99.10% (2192/2212) and 37.50% (12/32) for the interview and 45.83% (11/24), 99.73% (2206/2212) and 64.71% (11/17) for the combination of the two sources (both criteria fulfilled). **Conclusions:** Diagnoses based on information from the patient registry seemed to have reasonable validity, when the purpose is to study the etiology or health consequences of GDM. Adding information from interviews with the women themselves only increased validity marginally. This is to our knowledge the largest investigation on the validity of the GDM diagnosis.

P2-078

Determinants of Maternal Hyperglycaemia 6y after Delivery: Pune Maternal Nutrition Study (PMNS) Smita R. Kulkarni[1], Niranjan V. Joshi[1], Vaishali U. Deshpande[1], Parveen Bharucha[1], Chittaranjan S. Yajnik[1] and CHD Fall[2]; 1 Diabetes Unit, KEM Hospital and Research Center, Rasta Peth, Pune, India. 2 MRC Environmental Epidemiology Unit, Southampton, UK.

Background: Gestational diabetes was rare in young pregnant rural Indian women (PMNS) and plasma glucose concentrations were relatively low. We have followed these mothers and children and studied their glucose tolerance 6y later. This provides an opportunity to study the relationship between maternal glycemia in pregnancy (in normal range) and future risk of hyperglycemia. **Objective**: To study determinants of hyperglycaemia in mothers 6y after delivery. **Study Design**: The PMNS has information on maternal pre-pregnant size, socio-economic status, education and her nutritional and metabolic parameters during pregnancy. An oral glucose tolerance test (OGTT) and other measurements were repeated 6y after delivery. **Results**: Of 770 mothers studied during pregnancy, 7 were hyperglycaemic during pregnancy (3 impaired fasting glucose-IFG, 3 impaired glucose tolerant-IGT, 1 Diabetic-DM). Of the remaining 763 mothers, 650 were followed up 6y later at mean age of 27y. Fifty-two (8 %) of them were hyperglycaemic. Hyperglycaemic mothers were shorter (leg length 72.0 vs 74.0 cm, $p<0.01$), heavier (48.0 vs 44.0 kg, $p<0.001$), and more adipose (DXA fat % 30.0 vs 25.0, $p<0.001$) compared to normoglycemic mothers. They also had higher levels of other cardiovascular risk factors (cholesterol, triglycerides, HOMA insulin resistance) and had gained more weight since pregnancy (6.0 vs 3.0 kg, $p<0.0001$). There was no difference in education and socio-economic status. Before pregnancy (6y ago), currently hyperglycaemic mothers were centrally obese (waist circumference 62.8 vs 60.5 cm, $p<0.001$) and more adipose (sum of skinfolds 22.0 vs 20.0 mm, $p<0.001$) compared to the normoglycemic mothers but there was no difference in parity, family history of diabetes and weight gain during pregnancy. During pregnancy, these mothers were physically less active (Score 61 vs 75, $p<0.001$) and had higher blood pressure (115/64 vs 112/62 mmHg, $p<0.05$) and higher 2h plasma glucose concentration during OGTT (86 vs 78 mg% $p<0.001$). There was no difference in the incidence of pregnancy related events (anemia, PIH and caesarean section rate) in the two groups. Children of hyperglycaemic mothers had higher plasma glucose concentrations (fasting 96 vs 90 mg%, 2h OGTT 110 vs 96 mg%) at 6y compared to children of normoglycemic mothers. **Conclusions**: This is the first community-based study of incident hyperglycemia in rural India. We found a substantial incidence of hyperglycemia in young rural Indian women; this was predicted by short stature (leg length), lower physical activity and higher glycemia in pregnancy and higher weight gain after pregnancy. The predictive anthropometric and biochemical measures are far below any international 'risk factor' categories. These findings have implications for strategies to prevent maternal hyperglycemia in the Indian subcontinent.

P2-079

Impaired Beta-cell Development, Hyperglycemia, Hypoinsulinemia, Reduced GK Expression in Weanling Rats after Exposure to a High Fat Diet *in utero*
J. Louw[1], M.E. Cerf[1,2], K. Williams[1], C.J. Muller[2], D.F. Du Toit[2] and S.A. Wolfe-Coote[1] [1]Diabetes Research Group, Medical Research Council, Tygerberg, [2]Department of Anatomy and Histology, University of Stellenbosch, Tygerberg, South Africa

Background Normal beta-cell development is essential to ensure competent beta-cell function. A high fat diet (HFD) has been shown to adversely affect both beta-cell development and function. Reported here are the effects of a maternal HFD, during different periods of gestation, on weight, beta- and alpha-cell development and beta-cell function of weanling rats at three weeks of age. **Methods** Pregnant rats were maintained on a HFD for either the first (HF1) or second (HF2) or third (HF3) week of gestation, or throughout (HF1-3) gestation, followed by a standard laboratory diet for 21 days from birth of the pups until 3 weeks of age. On postnatal day 21, weights and circulating glucose and insulin concentrations were measured in the rat offspring that had been exposed to the HFD for periods during gestation. Pancreata were then excised and either processed for immunocytochemical examination and image analysis or snap-frozen for quantitative PCR. **Results** All of the weanlings had low body weights and were hypoinsulinemic. In HF1, HF2 and HF3 weanlings, hyperglycemia and a reduction in beta-cell number, beta- and alpha-cell size and glucokinase (GK) mRNA expression and immunoreactivity were evident. Apart from higher Pdx-1 mRNA expression in HF3 weanlings, Pdx-1 mRNA expression and immunoreactivity were normal. In HF1-3 weanlings, both beta-cell development and the expression of GK and Pdx-1 were normal. **Conclusion** A HFD *in utero*, for any single week of gestation, has a deleterious effect on beta-cell development and function in weanlings. This effect appears to occur independently of Pdx-1, while the reduced GK expression may contribute to the hypoinsulinemia and hyperglycemia.

P2-080

Indian Babies have Lower Adiponectin to Leptin Ratio in Cord Blood; Further Evidence for Intrauterine Origins of Insulin Resistance Himangi G Lubree[1], Chittaranjan S Yajnik[1], Sonali S Rege[1], Charudatta V Joglekar[1], Bhagyashree S Uradey[1], Jyoti A Deshpande[1], Smita R Kulkarni[1], Sadanand S Naik[1], Shaila D Kale[1], John S Yudkin[2]; 1. Diabetes Unit, KEM Hospital and Research Centre, Pune, India. 2. Diabetes and Cardiovascular Disease Academic Unit, Department of Medicine, University College London, UK

Background: We have shown that Indian babies and adults have higher body fat percent (adiposity) compared to the white Caucasian despite a smaller body mass index. Adiposity of Indians may contribute to the higher insulin resistance and risk of T2D. **Objective:** To compare adiposity and cord blood concentrations of adipocyte hormones (leptin, adiponectin) in newborns of normal glucose tolerant (NGT) and gestational diabetic (GDM) Indian mothers and those born to normal glucose tolerant (NGT) white Caucasian mothers. **Methods:** We measured plasma adiponectin and leptin concentrations in cord blood of babies born to 161 NGT and 168 GDM mothers in Pune, India and 78 babies born to NGT white Caucasian mothers in London, UK. Mothers and babies were measured soon after delivery. **Results:** Indian mothers were younger (27 y), shorter (1.53 m) and lighter (55 kg) compared to the white Caucasian mothers (32 y, 1.63 m, 70 kg respectively, $p<0.05$). GDM Indian mothers were older and more adipose compared to the NGT Indian mothers. Indian babies were born 7 days earlier and were lighter (2.8 kg) and shorter (48.1 cm) than the white Caucasian babies (40.4 wks, 3.6 kg, 51.0 cm respectively, $p<0.05$). The sum of skinfolds (triceps + subscapular) of the babies born to GDM Indian mothers was the highest (Indian GDM 10.8 mm, Indian NGT 8.3 mm, white Caucasians 9.9 mm). Cord plasma leptin concentration was higher in the babies of Indian GDM mothers compared to babies of NGT mothers (median 11.6 vs 5.6 ng/mL, $p<0.05$), there was no difference between the babies of NGT Indian mothers and babies of white Caucasian mothers (6.4 ng/mL). Cord plasma adiponectin concentration was lower in the babies of NGT mothers compared to those in babies of GDM mothers (18.3 vs 21.6 mg/L, $p<0.05$), there was no difference between those in the babies of GDM Indian mothers and white Caucasian mothers (25.3 mg/L). In each group leptin and adiponectin concentrations were related to neonatal size and adiposity, and also to each other except in the babies of the GDM mothers. Adiponectin / leptin ratio was the lowest in babies of GDM mothers and highest in white Caucasian babies (Indian GDM 3.8, Indian NGT 5.9, white Caucasians NGT 7.2). **Summary:** In comparison to the white Caucasian babies, the cord blood of Indian babies has lower adiponectin concentration in relation to leptin concentration. Babies born to Indian GDM mothers had the lowest adiponectin / leptin ratio. Our findings suggest that in addition to the higher relative adiposity of the Indian babies, their adipocyte hormone secretory pattern is also more conducive to producing a state of insulin resistance. Maternal diabetes exaggerates these abnormalities.

P2-081

Glucose Tolerance in Middle Age After Food Restriction During Gestation: The Dutch Famine of 1944-45 L.H. Lumey, Aryeh D. Stein; Henry S Kahn, Patricia A. Zybert; Gerard-Jan Blauw; Karin M. van der Pal – de Bruin. Mailman School of Public Health, Columbia University, New York NY, USA; Dept. of Global Health, Emory University, Atlanta GA, USA; Division of Diabetes Translation, Centers for Disease Control and Prevention, Atlanta, GA, USA; Leiden University Medical Center, Leiden, the Netherlands; TNO Quality of Life, Leiden, the Netherlands.

Background: Several studies in humans have reported associations between decreased fetal growth and the occurrence of type 2 (non-insulin dependent) diabetes in later life. We used the circumstances of the Dutch Famine of 1944-45, during which official rations were <900 kcal/day for 24 weeks, to assess whether generalized reductions of maternal food intake at specified stages of pregnancy were related to impaired glucose tolerance at age 59. **Methods**: We recruited three series of subjects: (1) exposed individuals born in one of three institutions in western Holland between January 1945 and March 1946, whose mothers were all exposed to the famine during or immediately preceding pregnancy; (2) unexposed individuals born in the same three institutions during 1943 or 1947, whose mothers did not experience famine exposure during this pregnancy; and (3) unexposed a same-sex sibling of subjects in series 1 or 2. Between 2003 and 2005, fasting glucose and insulin were obtained from 947 individuals. An oral glucose tolerance test was administered to 877 men and women with no history of diabetes. Blood samples were analysed for glucose and insulin. Homeostasis model assessment (HOMA) indices of insulin resistance (insulin*glucose/22.5) and beta cell function (insulin*20/glucose-3.5) were calculated from fasting glucose (mmol/L) and insulin (mU/L). We considered individuals with fasting glucose >= 5.6mmol/L (impaired fasting glucose) , 2 hr post-challenge glucose>= 7.8 mmol/L (impaired glucose tolerance) or a prior diagnosis of diabetes mellitus to have dysglycemia. We defined four (partially overlapping) windows of gestational exposure (gestational weeks (GW) 1-10; 11-20; 21-30; and 31 through delivery) based on exposure to a ration <900 kcal/day during the whole 10-week interval. Maternal preconception exposure status was characterized by a score representing cumulative weeks of exposure to reduced rations in the 6 months prior to conception. We used multiple linear regression to assess the joint effects of exposure in one or more periods of gestation, implemented in a hierarchical approach (GEE) to account for within-sibship

correlations. We adjusted initially for age and subsequently for height and waist circumference. **Results**: Among unexposed controls, mean fasting and 2-hr post-load glucose levels were 5.56 mmol/L (sd 1.19) and 5.86 mmol/L (sd 2.45); and 5.34 mmol/L (sd 1.21)) and 6.02 mmol/L (sd 2.08), in men and women, respectively. Dysglycemia was seen in 37.4% of men and 31.0% of women. Among men, no associations between any period of famine exposure before or during pregnancy and any of the glucose, insulin, or HOMA measures were significant at $p<0.10$. Among women, associations between famine exposure late in pregnancy and lowered fasting insulin ($p < 0.05$), and between exposure early in pregnancy and increased fasting glucose and 2 hr glucose (both $p < 0.10$) attained significance. There appears to be little pattern in these associations. These results were not affected by adjustment for height and waist circumference and were robust to the inclusion or exclusion of individuals with a prior history of diabetes. **Conclusions**: In this population, prenatal famine exposure was not associated with fasting glucose or with HOMA indices of insulin resistance or beta-cell dysfunction. These results do not confirm previously reported findings from the Netherlands that prenatal exposure to famine, especially during late gestation, is linked to decreased glucose tolerance in adults.

P2-082

Higher Hb A_{1c} Levels Among Brazilian Males with Restricted Fetal Growth

Aydin Nazmi[1], Sharon R Huttly[1], Cesar G Victora[1], Rosangela C Lima[1], Paulo R Post[1], Jacqueline WL Elizalde[1], Branca MC Gerson[4 1] Federal University of Pelotas, Post-Graduate Program in Epidemiology, Avenida Duque de Caxias, Pelotas, RS, 96030-002, Brazil [1] London School of Hygiene and Tropical Medicine, Nutrition and Public Health Intervention Research Unit, Keppel Street, London WC1E 7HT, UK [1] Federal University of Pelotas, Department of Microbiology and Parasitology, Avenida Duque de Caxias, Pelotas, RS, 96030-002, Brazil [1] Brazilian Army, Tuiuti Regiment – 8th Motorized Infantry Brigade, Avenida Duque de Caxias Pelotas, RS 96030, Brazil

Objective This study aimed to determine if there was a difference in Hb A_{1c} levels among adolescent males according to their fetal growth rate. **Background** The fetal origins of adult disease hypothesis has been widely studied in many populations and age groups. The main premise is that nutritional deprivation *in utero* affects fetal development and contributes significantly to the determination of diseases later in life associated with the metabolic syndrome. The thrifty phenotype hypothesis proposes that intra-uterine, as well as post-natal environmental influences contribute to type 2 diabetes by permanently programming metabolic responses. Most studies, however, have been carried out in high-income countries. **Methods** The 1982 Pelotas, Brazil birth cohort provided the subsample for this study. We performed glycosylated hemoglobin tests on male adolescents aged 18 years, 88 of whom were born low birthweight and 174 born appropriate birthweight, all of whom had been followed up since birth. Hb A_{1c} levels were compared according to subjects' birthweight-for-gestational age z-score quartile with adjustment for potential confounding factors. **Results** Complete data were available on 197 individuals. There was a negative trend in mean %Hb A_{1c} with increasing birthweight-for-gestational age (mean %Hb A_{1c} for each quartile: 5.35, 5.27, 5.18, and 5.08; p=0.009). Adjusting for family income and mother's education (measured in 1982), the association remained significant at p=0.01. **Conclusion** We have shown that being born small for gestational age is associated with higher mean Hb A_{1c} levels in male adolescents. It is suggested that individuals born in the lower birthweight for gestational age quartiles experienced reduced intrauterine growth as a result of fetal undernutrition and were therefore physiologically programmed to be more susceptible to hormonal and metabolic abnormalities. These findings agree with similar studies that indicate higher blood glucose concentrations among adults and children who were small at birth. We highlight the need for prospective birth cohort studies in both high and low-income countries to allow data to be examined in the wider perspective of the life course, taking into account maternal, growth, and environmental variables.

P2-083

Higher Insulin Resistance in Non-obese 8 Year Old Indian Girls Predicts Earlier Menarche

Madhumati S Otiv, Ashish R Bavdekar, Sheila A Bhave, Anjali S Mote, Vaishali A Madkaikar, Anand N Pandit, Sadanand S Naik, Chittaranjan S Yajnik, Charu V Joglekar, Caroline HD Fall, Department of Pediatrics and the Diabetes Unit, King Edward Memorial Hospital, Pune 411011, India, Medical Research Council, Environmental Epidemiology Unit, University of Southampton, UK.

Background: Previously we demonstrated an inverse relationship of birth weight with post glucose load 30 min glucose at 4 years of age in an urban Indian cohort (n=190). We did a follow up study of this cohort at 8 years of age with additional 287 children (n = 477, boys =255, girls = 222) for their anthropometry, HOMA, cholesterol, triglycerides, HDL, BP, and leptin. This study demonstrated that children with the most adverse cardiovascular risk profiles were born light but were relatively fat and tall at 8 years. This cohort has been annually followed up from 12 years of age and has now reached ages 16-17 years. **Aim:** This paper describes relationship of insulin resistance at 8 years with pubertal development in girls from the same cohort. **Methods:** We enrolled the entire 8 year cohort for annual anthropometry, sexual maturity (SMR, Tanner staging) and recorded age of menarche. Bone age (Grulich and Pyle) was done at 12 years. Peak height velocity was defined as maximum linear growth between 2 consecutive annual records in a child who has had at least 5 records during the study period. Sum of skinfold (SSF) was defined as sum of subscapular, triceps, biceps and suprailiac skinfold. Onset of puberty was defined as stage II of SMR, and completion of puberty as stage V of SMR. This analysis pertains only to girls who have completed their puberty (n=165, 74.32%). Boys have been excluded from this analyses as only 113 (63.52%) have completed their puberty. **Results:** The mean age at the most recent visit was 16.51 (SD 0.74) years. Age of onset of puberty was available only in 46 (20.72%) girls who had not entered puberty at the beginning of the study; peak height velocity was available on 136 (83.95%) girls. The mean age of menarche recorded for 163 (98.78 %) girls was 12.77 (SD 1.06) yrs. HOMA (r -0.188, p=0.02), leptin (r -0.225, p=0.005), fat mass (r -0.366, p=0.000), BMI (r -0.330, p=0.000), weight (r -0.388, p=0.00), height (r -0.267, p=0.001), waist circumference (r -0.315, p=0.00), SSF (r -0.358, p=0.000) at 8 years and bone age at 12 years (r -0.551, p= 0.00) was inversely related to age of menarche. Multivariate analysis showed that advanced skeletal maturity (ß= -0.477), higher waist circumference (ß= -0.199), and higher HOMA (ß= -0.148) at 8 years independently predicted earlier menarche (adjusted R^2 = 35 %). BMI (r -0.235, p=0.002), weight (r -0.276, p=0.00), height (r -0.181, p=0.020), waist (r -0.183, p=0.018), fat mass (r -0.173, p=0.26) at 8 years was inversely related to the age of completion of puberty. Multivariate analysis showed that earlier age at peak height velocity (ß 0.358) and age at menarche (ß=0.174) predicted early completion of puberty (adjusted R^2 =14.6 %). **Conclusions:** Insulin resistance and central adiposity at 8yrs, associated with pre-pubertal height spurt in non-obese urban Indian girls predicted earlier menarche. Earlier menarche and accelerated linear growth predicted earlier completion of puberty.

P2-084

The Effect of Breast Feeding on Type 2 Diabetes and its Precursors: A Systematic Review of Published Evidence

Christopher G Owen, Richard M Martin, Peter H Whincup, George Davey-Smith, and Derek G Cook; Division of Community Health Sciences, St George's, University of London, Cranmer Terrace, London, UK, SW17 0RE. Department of Social Medicine, University of Bristol, Canynge Hall, Whiteladies Road, Clifton, Bristol, UK, BS8 2PR.

Background: It has been suggested that infant feeding pattern may influence susceptibility to diabetes in later life. The purpose of this study was to use the published literature to examine the influence of initial infant feeding on type 2 diabetes, and markers of hyperglycaemia and insulin resistance. **Methods:** A systematic review of published studies investigating the association between infant feeding, diabetes and its precursors, using MEDLINE, EMBASE, and Web of Science databases, supplemented with manual searches. Odds ratios of type 2 diabetes, and the ratio of geometric means in fasting insulin expressed as a percentage difference (children and adults without diabetes), mean differences in blood glucose and serum insulin (for infants only), among initially breast-fed subjects compared to subjects formula-fed, were pooled using fixed effects models (ratios less than one imply that breast feeding is associated with lower insulin levels and prevalence of diabetes compared to formula feeding, breast fed minus formula fed for mean differences). To differentiate acute from long-term influences of breastfeeding, studies in infants were examined separately from those in children and adults. **Results:** Among adults (6 studies, 76,211 subjects), those who were breast fed showed a lower risk of type 2 diabetes in later life than those who were formula fed (odds ratio 0.61, 95% CI 0.43 to 0.86, P=0.005, χ^2 test for heterogeneity P=0.28). In 2 of these studies in which adjustment for potentially important confounders was carried out, the association persisted. In children and adults without diabetes (5 studies, 4267 subjects) those who were breast fed had marginally lower fasting insulin than those formula fed (-4%, 95% CI –9% to 0%, P = 0.07). In infants (7 studies, 291 subjects), breast-fed subjects had lower mean blood glucose than those formula fed (mean difference -0.17 mmol/L, -0.28 to -0.05, P=0.005) and lower mean insulin levels (7 studies, 291 subjects, mean difference -2.86 pmol/L, -5.76 to 0.04, P=0.054). **Conclusions:** Breast feeding appears to be associated with a marked decrease in the risk of type 2 diabetes in adults and possibly with lesser degrees of insulin resistance in childhood and adult life. It also appears to have appreciable short-term effects on glucose and insulin metabolism in early life. It is possible therefore that breast feeding could have long-term protective effects against diabetes, which could be mediated by metabolic programming. However, the studies on which adult data are based involve unusual populations and the wider relevance of these findings remains uncertain. More evidence on the long-term relationship between infant feeding and diabetes in other adult populations is needed to substantiate these findings.

P2-085

Effect of Metyrapone on Circulating Cortisol and Glucose Homeostasis in the Guinea Pig

Sanita Grover[1, 2], Cathie L. Coulter [1], Melissa R. Walker[2], Karen L. Kind [2], Jeffrey S. Robinson [2] and Julie A. Owens[2]; School of Molecular and Biomedical Science, Discipline of Physiology[1], Department of Obstetrics and Gynaecology[2], The University of Adelaide, Adelaide, SA, 5005 Australia

Background Low birth weight in babies has been associated with an increased risk of developing the insulin resistance syndrome and coronary heart disease as an adult. Insulin resistance (IR) syndrome has many similarities with Cushing's syndrome and therefore it has been suggested that increased hypothalamic pituitary adrenal axis activity may underlie the development of IR following poor growth before birth. In support of this, low birth weight in humans is also associated with increased plasma cortisol concentrations in adult life. In the guinea pig fetal growth restriction results in adult IR, glucose intolerance, and increased blood pressure and with elevated salivary cortisol concentration. However, whether prenatally induced increases in circulating cortisol concentrations contribute to the IR, glucose intolerance and diabetes in adult life remains to be determined. Metyrapone decreases circulating cortisol in the guinea pig within two days through inhibition of 11ß-hydroxylase, a key enzyme involved in cortisol biosynthesis. The aim of this study was to determine the impact of variable

birth weight and circulating cortisol concentrations on fasting plasma glucose and glucose tolerance in the young adult guinea pig, before and after metyrapone treatment. **Methods** Vascular catheters were implanted at 100 days of age in young adult male guinea pigs of known size at birth. An intravenous glucose tolerance test (IVGTT) (0.5g/kg at 0 min; blood was sampled frequently between –10 and 210 min) was performed at 115 days of age after an overnight fast. Metyrapone (100mg/kg/day IV) or vehicle was administered once daily in the morning for three consecutive days and a second IVGTT performed. Plasma cortisol was measured by specific radioimmunoassay and blood glucose by Glucometer. Glucose tolerance was calculated as the area under the glucose curve (GAUC). **Results** Birth tertile altered fasting blood glucose (FBG) (p=0.08), with FBG increasing with decreasing abdominal circumference (AC) (r=-0.504, p=0.007), head width (HW) (r=-0.389, p=0.033) and H length (HL) (r=-0.571, p=0.002) at birth, at first IVGTT. Glucose tolerance was increasingly impaired with decreasing crown rump length (r=-0.291, p=0.089), HW (r=-0.346, p=0.053) and HL (r=-0.401, p=0.029) at birth. Birth weight tertile also altered plasma cortisol (p=0.1), with levels in low birthweight 1.5 to 2.7 times those in average and high birth weight animals. Plasma cortisol also correlated positively with glucose tolerance at first IVGTT (p=0.023). In general these effects of size at birth on glycaemia and plasma cortisol were strengthened following vehicle treatment. Metyrapone reduced fasting plasma cortisol compared to vehicle (p=0.07) and by -47% compared to that at first IVGTT (p=0.029). Repeat IVGTT impaired glucose tolerance (-84%) (p=0.006) and this was ameliorated by metyrapone (-16%) (p=0.09), particularly in animals of low and average birth weight. **Conclusion** Prenatally induced increases in circulating cortisol accounts for much but not all the impaired glucose tolerance observed following fetal growth restriction in the young adult guinea pig of small size at birth.

P2-086

Gestational Diabetes in Iran: Incidence, Risk factors and Pregnancy Outcomes

Babaee Gh. (Ph.D), M. Parsinia, (MSc) P. Ashkvari (MSc) Nursing & Obstetrics Faculty, Islamic Free University, Karaj-Iran Email: babaee99@yahoo.com

The objective of this study was to determine the incidence of gestational diabetes mellitus (GDM) and compare fetal, maternal and neonatal complications amongst women with GDM and pregnant women with normal glucose tolerance in an urban Iranian population. In a prospective cohort study, universal screening for gestational diabetes mellitus was performed for 1310 pregnant women who were referred from private clinics and community health care centers to Fatemiyeh Hospital in Shahrood City. Screening was performed with a 50-g oral Glucose Challenge Test (GCT) with 130mg/dl cut–off point, then a diagnostic 100-g Oral Glucose Tolerance Test (OGTT) was done according to Carpenter and Coustan criteria. The incidence of GDM was 4.8%. There were differences in risk factors: age>30yr, family history of diabetes, obesity, previous macrosomia, glycosuria between the two groups (P<0.001). Women with GDM had a higher rate of stillbirth (P<0.001; odds ratio 17.1, 95% CI=4.5-65.5), hydramnios (P<0.001; odds ratio 15.5, 95% CI=4.8-50.5), gestational hypertension (P<0.001; odds ratio 6, 95% CI=2.3-15.3), macrosomia (P<0.05; odds ratio 3.2, 95% CI=1.2-8.6) and caesarean section (P<0.001). We have found that the incidence of GDM in an urban Iranian population is similar to developed countries. Complications were more common in the GDM group than in the normal group and outcomes for women with persistent diabetes postpartum were particularly poor. We recommend screening for GDM in Iran, but further evaluation of selective screening and cost effectiveness will need to be performed. Measures to improve the outcome of GDM pregnancy will also need to be addressed in the future.

P2-087

Differences in the Relationship Between Fetal Life and Components of the Metabolic Syndrome Between Men and Women

Mark S. Pearce, Nigel C. Unwin, Louise Parker, on behalf of the Newcastle Thousand Families Study. *School of Clinical Medical Sciences, University of Newcastle upon Tyne, Sir James Spence Institute, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, United Kingdom.*

Background Numerous studies have shown relationships between birth weight and health in middle age. What is less clear is how the developmental origins of adult disease may vary between men and women. We investigated interactions between gender and birth weight on a components of the metabolic syndrome, using data from the Newcastle Thousand Families Cohort Study which began when all 1142 births in May and June 1947 were recruited into the study. **Methods** Detailed information was collected prospectively on factors including birth weight and gestational age. Birth weights were adjusted for gestational age and gender. At age 50 years, 412 study members (180 men and 212 women, representative of the original cohort) attended for a clinical examination. A central metabolic syndrome (CMS) score was derived using a principal components approach, incorporating BMI, waist:hip ratio, systolic and diastolic blood pressure, serum HDL cholesterol and triglyceride and both fasting and 2 hour post challenge plasma glucose and serum insulin concentrations. Fasting and 30min serum insulin and glucose levels were used to derive HOMA-IR and insulin secretion. Interactions between gender and birth weight on CMS score and insulin, glucose, lipid and blood pressure variables, log-transformed where appropriate, were assessed using linear regression. **Results** There was a significant interaction between birth weight and gender for log 2-hour glucose (p=0.023) for which a birth weight association was seen in men (r=-0.06, 95% CI -0.10, -0.02, p=0.006), but not in women (r=-0.001, p=0.933). For log fasting glucose, an association with birth weight was again seen in men (r=-0.02, 95% CI -0.03,-0.001, p=0.031), but not in women (r=0.001, p=0.884) and the interaction between birth weight and gender did not quite reach statistical significance (p=0.08). Similar results were seen for HDL cholesterol (interaction p=0.07), but there was less evidence of an interaction for triglycerides (interaction p=0.13), with significant relationships between birth weight and both outcomes restricted to males only for both. Although interactions were not noted for blood pressure (p>=0.5), the association between birth weight and log systolic blood pressure was restricted to the women in the study (r=0.015, 95% CI -0.03,-0.0005, p=0.043). Birth weight was negatively associated with CMS score in men (r=-0.15, 95% CI -0.32, 0.02, p=0.08), but little correlation was seen in women (r=0.01, 95% CI -0.12, 0.13, p=0.88) and the interaction term was not significant (p=0.14). Similar results were seen for HOMA-IR and insulin secretion, where the interaction terms again failed to reach significance (p=0.14 and p=0.28 respectively). **Conclusions** Gender differences in lifecourse influences on adult health have previously been suggested for this cohort. This study suggests that the influence of birth weight on components of the metabolic syndrome may vary between men and women. It is possible that the lack of further significant interactions is due to a lack of statistical power, particularly as the differences in regression coefficients and significance levels are apparent between the genders for some of the outcomes considered.

P2-088

Place of Birth and Risk of Metabolic Syndrome in Young British Pakistani Women

Tessa M. Pollard[1], Nigel Unwin[2], Colin Fischbacher[3] and Jagdip K. Chamley[2]; [1]Department of Anthropology, University of Durham, UK, [2]Paediatric and Lifecourse Epidemiology Research Group, Medical School, University of Newcastle, UK, [3]ISD, Edinburgh, UK.

Background A high prevalence of metabolic syndrome, type 2 diabetes and coronary heart disease is found in people of South Asian origin living in affluent countries. There is evidence that babies born in South Asia are small, but have a relatively large fat mass, with central fat patterning, and it has been suggested that this leads to a greater vulnerability to metabolic syndrome on exposure to lifestyle risk factors in later life. One implication is that future generations born in countries such as the UK, where babies are generally larger and have a lower proportion of body fat, may be less susceptible to the development of metabolic syndrome. As part of a study of 20-40 year old women of Pakistani and European origin living in the UK we tested the prediction that features of metabolic syndrome would be found at highest levels in women born in Pakistan, with lower levels in Pakistanis and Europeans born in the UK. **Methods** We recruited participants from urban areas of north-east England using convenience sampling, excluding women with diagnosed diabetes or who were pregnant, breast-feeding or using oral contraceptives. Most women (26/30) born in Pakistan migrated to the UK after the age of 16. Blood samples were taken after an overnight fast. Suprailiac and subscapular skinfolds were summed. We applied a logarithmic transformation to positively skewed variables.

Results

	Pakistani born Pakistan (N=30)	Pakistani born UK (N=30)	European born UK (N=25)	F	p	Eta^2
Age (years)	31.1 (29.3, 33.0)	29.2 (27.3, 31.0)	34.6 (32.5, 36.6)	7.68	<0.001	0.16
Height (m)	1.57 (1.55, 1.59)	1.60 (1.58, 1.62)	1.63 (1.62, 1.66)	8.85	<0.001	0.18
BMI	25.6 (23.8, 27.4)	24.1 (22.4, 25.8)	26.8 (24.8, 28.8)	2.12	0.13	0.05
Waist (cm)	79 (74, 83)	75 (71, 79)	83 (78, 88)	3.22	0.05	0.07
Triglycerides (mg/dL)	108 (89, 132)	77 (63, 95)	90 (72, 112)	2.84	0.06	0.07
Truncal skinfolds (mm)	48 (42, 56)	41 (36, 48)	40 (33, 47)	1.97	0.15	0.05
HDL (mg/dL)	50 (46, 54)	65 (61, 69)	57 (53, 62)	13.41	<0.001	0.25
Fasting glucose (mg/dL)	90 (87, 93)	85 (81, 87)	87 (84, 90)	3.09	0.05	0.07
Fasting insulin (mU/l)	10.4 (7.7, 13.8)	9.1 (6.9, 12.2)	7.2 (5.3, 9.9)	1.45	0.24	0.04
HOMA-IR	2.31 (1.70, 3.13)	1.91 (1.40, 2.59)	1.42 (1.01, 2.01)	2.19	0.12	0.06

Table 1. Comparison of group means (95% confidence intervals) for the main variables. Geometric means and confidence intervals reported for log transformed variables. BMI=body mass index, HDL=high density lipoprotein, HOMA-IR=homeostatis model assessment – insulin resistance. The results (Table 1) showed that women born in Pakistan were shortest, reflecting a poorer early environment. Pakistan-born women had the highest levels of fasting triglycerides, glucose, insulin and HOMA-IR and the lowest levels of HDL, although only two of these effects were statistically significant. The European group had the highest waist circumference, but when age and BMI were added to the models the group effect for waist circumference was greatly reduced (F=0.87, p=0.42) and the effect for truncal skinfolds greatly increased (F=8.7, p<0.001), with the highest adjusted mean for truncal skinfolds seen in women born in Pakistan. The differences in the other variables were affected to only a small extent by adjustment for age and BMI. **Conclusions** Our findings provide some support for the suggestion that women born in Pakistan and living in the UK have greater risk of metabolic syndrome than women born in the UK. Our sample was small and unrepresentative and more work is needed to replicate this finding and examine the relative contributions of environments in early and later life. Funding: Newcastle Health Care Charity.

P2-089

Onset and Duration of Overweight over 40 Years of Follow-up from Birth in Relation to Risk of Type 2 Diabetes. Chris Power, Claudia Thomas; *Centre for Paediatric Epidemiology and Biostatistics, Institute of Child Health*, 30 Guilford Street, London WC1N 1EH, UK

Background. Weight gain in adult populations is known to increase the risk of type 2 diabetes. Evidence regarding long-term effects of overweight in childhood on diabetes risk in adulthood is scarce and is of particular concern in view of recent increases in some developed countries in childhood obesity. Identification of possible critical periods in the development of overweight has therefore been elusive in relation to risk of type 2 diabetes. Thus, risks for children who are relatively fat in childhood but who are less so in adulthood are not well-documented. We examined BMI over several ages in childhood and adulthood, identifying onset and duration of overweight, in relation to diabetes risk in mid-life. **Methods.** Prospective data on BMI during childhood (ages 7, 11 and 16y) and in adulthood (23, 33 and 44y) for participants in the 1958 British birth cohort were analysed with risk of type 2 diabetes at age 44y as the outcome. Blood samples (n=7892) obtained in 2002-2004 during a clinical assessment were assayed for glycosylated haemoglobin (HbA1c). Analyses were conducted excluding those with type 1 diabetes (n=54). Type 2 diabetes was defined as either undiagnosed (HbA1c =7%) or previously diagnosed type 2 diabetes. Participants reported whether they had been told that they had "non-insulin diabetes that is controlled by diet or tablets" (n=67). Diabetes risk was defined as those with diagnosed or undiagnosed type 2 diabetes plus those with hyperglycaemia (HbA1c 6-6.99%). Overweight in childhood was defined using International standards[1] and in adulthood, using the WHO cut-off BMI =25 kg/m^2. Logistic regression was used to estimate the odds ratios for associations between age of onset of overweight, duration of overweight, and diabetes risk, adjusted for sex. The final sample size for analyses depended on the completeness of BMI data at each age. **Results**. Prevalence of type 2 diabetes was 1.7% by age 44y and a further 2% had evidence of hyperglycaemia (HbA1c 6-6.9%). Overweight was mostly of adult onset (21.5% in mid-adulthood [44y] and 26.3% in young adulthood [23 or 33y]), 2.2% with onset in adolescence (16y) and 13.5% in childhood (7 or 11y). Only 2.2% of participants were overweight in childhood or adolescence and not thereafter. Compared to the reference category (33.9% normal weight at all life-stages, with a diabetes prevalence of 0.4%) the risk of diabetes was greatest for participants with onset of overweight in childhood or adolescence (OR=10.8, 95%CI=6.6-17.6), that is those with the longest duration of overweight. Onset of overweight in young adulthood conferred an increased risk (5.7, 1.4-3.5), but for those with recent onset or overweight only in childhood diabetes risk was not elevated (1.6, 0.9-3.1 and 0.81, 0.1-6.1 respectively). In additional analyses of number of ages an individual was classified as overweight, our finding of increasing diabetes risk with increasing duration of exposure to overweight was confirmed. Similar patterns for onset and duration of overweight were found using HbA1c as a continuous outcome. **Conclusions.** Earlier onset of overweight, and thus, longer duration of overweight, is associated with an increased risk of type 2 diabetes. This finding has implications for the increasing proportion of children who are overweight. However, diabetes risk in mid-adult life appears to be averted in overweight children who do not become overweight adults. Similarly, middle-aged adults with no prior history of overweight had no excess risk of diabetes compared to individuals who had never been overweight. Effects of overweight on type 2 diabetes risk are cumulative, thus prevention of overweight at any age can lessen the risk of development of type 2 diabetes.

1. Cole, TJ et al. BMJ, 2000, 320: 1240-3.

P2-090

Obesity from Cradle to Grave - Childhood Growth and Metabolic Syndrome in Obese Men and Women Minna Salonen, Eero Kajantie, Tom Forsen, Hilkka Ylihärsilä, Clive Osmond, David JP Barker, Johan Eriksson; National Public Health Institute, Department of Epidemiology and Health Promotion, Finland, MRC Epidemiology Resource Centre, UK, University of Southampton, Developmental Origins of Health and Disease Division, UK

Background. Upward trends in the prevalence of obesity have occurred in most populations worldwide. Obesity is generally associated with a large number of CVD risk factors and increased morbidity and mortality. Study design. The study is a part of the Helsinki Birth Cohort Study including 8760 people born 1934 – 1944. This unique cohort has detailed birth and growth data including birth measurements, as well as data on placental weight, gestational age and maternal size. Information available from child welfare and school health records includes serial growth measurements during infancy and childhood. Information about weight at 20 years and family history of diabetes was available from a questionnaire. 2003 subjects were randomly selected for a detailed clinical study for assessment of glucose and lipid metabolism, body composition and life style factors. All 499 obese individuals (BMI $\geq$ 30 kg/m^2) were included in this sub-study. The aims of the study. To study specific growth patterns associated with low metabolic risk profile in obese subjects. **Methods.** Tests for trends were based on multivariate linear and logistic regression, adjusted for age at examination and gender. We converted each height, weight and BMI measurement for each child to a Z-score using the method of Royston. These Z-scores were assessed on birthdays by interpolation and back-transformed. History of maternal and paternal diabetes was included in analysis. **Results.** The prevalence of the metabolic syndrome was 80 % in the obese study group according to the IDF 2005 criteria. The cumulative incidence of the metabolic syndrome decreased with increasing childhood weight and BMI at ages 2 years as well as between 7 and 11 years of age. Characteristics of the study group are shown in the table. Height was not related to the occurrence of the metabolic syndrome. There were no significant associations between birth size and the metabolic syndrome. **Conclusions.** The metabolic syndrome is commonly associated with obesity, although one fifth of these obese individuals were metabolically normal. In obese 60 – 70-year-old men and women certain patterns of growth during childhood seems to be protective against the metabolic syndrome.

Table. Characteristics of study group (n=499).

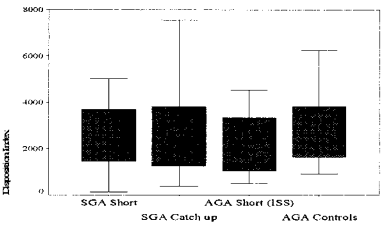
	Metabolic Syndrome + (n=400, BMI $\geq$ 30)	Metabolic Syndrome - (n=99, BMI $\geq$ 30)			
	Mean SD	Mean SD	Mean diff.	95% CI	p-value
Birth weight (g)	3453 520	3470 457	16.0	-96.1 to 127.9	0.780
BMI at 2 years (kg/m^2)	16.6 1.2	17.0 1.3	0.3	0.04 to 0.59	0.024
BMI at 7 years (kg/m^2)	15.7 1.3	16.4 1.1	0.6	0.40 to 0.90	0.000
BMI at 11 years (kg/m^2)	17.5 1.8	18.2 1.7	0.7	0.27 to 1.06	0.001
BMI at 20 years (kg/m^2)	23.0 2.7	23.5 2.9	0.6	-0.02 to 1.21	0.057
Current BMI (kg/m^2)	34.1 4.1	33.0 3.0	-1.1	-1.79 to -0.35	0.004
Lean body mass	60.9 7.0	59.1 5.6	-1.8	-3.11 to -0.48	0.008
Body fat (%)	37.1 4.9	36.5 3.7	-0.6	-1.48 to 0.03	0.200
Mother's BMI *) (kg/m^2)	26.9 3.2	27.3 3.2	0.4	-0.36 to 1.13	0.311
Family history of DM (%)	18.1	15.0		#) 0.746	

Values are adjusted for gender and age at clinical examination.
*) Mother's BMI before delivery
#) chi square-test

P2-091

The Effect of Birth Size, Adult Size and Body Composition on Insulin Sensitivity and Secretion in Young Adults: The PROGRAM Study Annabelle S. Slingerland[1], Theo Stijnen[2], Eric S. Krenning[3], Patty Oosterbeek[1], Lisette Holl[1], Alwine A. Hellingman[1], A.C.S.Hokken-Koelega[1] [1]Department of Pediatrics, Division of Endocrinology, Erasmus Medical Center-Sophia Children's Hospital [2]Department of Epidemiology and Biostatistics [3]Department of Nuclear Medicine Erasmus Medical Centre, Rotterdam, the Netherlands

Background *Low birth weight is associated with a 35% increase in type 2 diabetes mellitus risk and, in addition, postnatal catch up in weight and short adult height per* se might increase this risk. At this moment, the relative contribution of birth size, adult size and body composition to the development of type 2 diabetes has still not been disentangled. The PROGRAM-study was designed to anwer these questions. Answers are required before implementing preventive measures or intervention. **Methods** The PROGRAM study tested one hundred and forty nine 21 year old subjects and four predefined groups that met specific criteria for birth length and adult height: 1. Small for Gestational Age(SGA)-Short in adulthood 2. SGA- Catch up: Normal height in adulthood 3. Appropriate for Gestational Age (AGA) – Short in adulthood (Idiopathic Short Stature) 4. AGA-Controls (normal birth and adult height). Insulin sensitivity, insulin secretion and their product Disposition Index were determined from a Frequently Sampled tolbutamide modified Intravenous Glucose Tolerance test. DXA-scanning was used to assess body composition. **Results** Insulin sensitivity and the disposition index as most important determinants for type 2 diabetes, were comparable for all groups (Table). Multiple regression showed that insulin sensitivity was strongest predicted by waist circumference (R^2=0.32, ß=-0.6, p<0.001) Birth size and Adult height had no independent effect on insulin sensitivity or on disposition index.



Conclusions At the age of 21 years, SGA and ISS subjects have no increased risk of type 2 diabetes mellitus as their disposition indices were not decreased compared to age-matched Controls. Adult waist circumference best predicted insulin sensitivity while birth size and adult size had no predictive value. Rather than attributing increased risk of type 2 diabetes mellitus to low birth size or short adult height, measures should primarily focuss on preventing a large waist circumference in all groups.

P2-092

Insulin Sensitivity and Secretion in Young Adults and Symptoms of Preeclampsia in Their Mothers Katarzyna Szamotulska [1], Dorota Szostak-Wegierek [2]
[1)] Department of Epidemiology, National Research Institute of Mother and Child, Warsaw, Poland [2)] Department of Nutrition Related Diseases, National Food and Nutrition Institute, Warsaw, Poland.

Background: Results of epidemiologic studies suggest inverse relationship between glucose intolerance in adult life and size at birth. Aim: To investigate the relationship between glucose and insulin metabolism in young adults and chosen complications of maternal pregnancy influencing birth weight. **Methods**: The study group consists of persons aged 24-29 years, whose mothers participated in the prospective cohort study of risk factors of low birth weight during pregnancy conducted at the National Research Institute of Mother and Child in Warsaw in 1974-77. Original structured questionnaires from this study collected at the first and second visit during pregnancy and after delivery have been stored for 1912 mother until now. All mothers were asked for help in inviting their children to physical examination and fasting blood samples testing at the National Food and Nutrition Institute in 2000-2004. 495 children agreed to participate (214 men and 281 women). Multifetal pregnancies and diabetic women were originally excluded from the study in the 70-ties. Men and women taking blood pressure, lipids or glucose lowering drugs and women taking oral contraceptives were additionally excluded from the current study. Fasting glucose, fasting insulin, HOMA-IR index and HOMA-βC index were used for the analysis of glucose and insulin metabolism. Maternal anemia during pregnancy, vaginal bleeding during pregnancy, urinary tract infections, placental insufficiency, cardiovascular disease, preeclampsia (hypertension and/or proteinuria and/or oedema in late pregnancy) were taken into account. Linear regression analysis on transformed parameters of glucose and insulin metabolism adjusted for current body mass index was applied. **Results**: Maternal anemia during pregnancy, vaginal bleeding during pregnancy, urinary tract infections, placental insufficiency and cardiovascular disease were not statistically related to current parameters of glucose and insulin metabolism in young adult children of both sexes. Preeclampsia was not statistically related to fasting glucose concentration, in both sexes as well. However, preeclampsia was statistically significantly related to fasting insulin, HOMA-IR and HOMA-βC in men, and not statistically significantly – in women. In young male adults – children of preeclamptic women, fasting insulin was 11.1. mU/l and in young male adults – children of not preeclamptic women, fasting insulin was 9.0 mU/l (p=0,024). HOMA-IR levels were 2.6 and 2.1 (p=0,041), respectively and HOMA-βC were 124 and 97 (p=0,016), respectively. In young female adults – children of preeclamptic women, fasting insulin was 7.9 mU/l (p=NS) and in young female adults – children of not preeclamptic women, fasting insulin was 6.9 mU/l (p=NS). HOMA-IR levels were 1.8 and 1.5 (p=NS), respectively and HOMA-βC were 104 and 91 (p-NS), respectively. **Conclusions**: Children of preeclamptic women may be at risk of impaired insulin sensitivity and insulin secretion in adult life.

P2-093

Undernutrition During Pregnancy Leads To Obesity Without Insulin Resistance NM Thompson[1], MH Vickers[1], SO Krechowec[1], J Miles[1], R Shankar[2], A Norman[1], SS Donkin[3], BH Breier[1]. National Research Centre for Growth and Development and Liggins Institute[1], University of Auckland, Auckland, New Zealand, Indiana University[2] and Purdue University[3], West Lafayette, Indianapolis , USA.

Offspring of rats undernourished during pregnancy develop obesity, fasting hyperinsulinaemia and hyperleptinemia. However, effects on insulin sensitivity in adult life are not known. Virgin Wistar rats were time-mated and assigned to receive chow either *ad-libitum* (AD) or at 30% of *ad-libitum* intake (UN) throughout pregnancy. Male offspring were fed either *ad-libitum* chow diet (C), a regime of calorie restriction (70% of C (CR)) or high fat diet (HF) *ad-libitum* from weaning until study completion. At 263±2 days of age hyperinsulinaemic-euglycemic clamps were performed under halothane anaesthesia (n=6/group). A parallel cohort of animals was used for endocrine measurements. Adult UN offspring were obese (P<0.05) (% retroperitoneal fat pads ADC 2.7±0.2, ADCR 1.1±0.1, ADHF 5.7±0.3, UNC 3.4±0.4, UNCR 1.6±0.1, UNHF 6.1±0.3), hyperinsulinaemic (P<0.001) (fasting plasma insulin(μg/L) ADC 0.7±0.1, ADCR 0.4±0.1, ADHF 0.9±0.1, UNC 3.5±1.0, UNCR 1.1±0.3, UNHF 3.8±0.7) and hyperglycaemic (P<0.001) (fasting plasma glucose (mmol/L) ADC 8.6±0.3, ADCR 7.2±0.3, ADHF 7.6±0.2 UNC 9.7±0.3, UNCR 7.6±0.2, UNHF 9.0±0.3) compared to AD offspring. HF animals showed decreased (P<0.05) sensitivity to insulin compared with those fed standard chow (glucose infusion rate/plasma insulin ((mg/kg/min)/μg/L) ADC 0.32±0.02, ADCR 0.47±0.09, ADHF 0.2±0.01, UNC 0.31±0.04, UNCR 0.45±0.05, UNHF 0.18±0.44). In contrast, CR throughout postnatal life increased insulin sensitivity (P<0.01). The level of maternal nutrition during fetal development had no independent effect on insulin sensitivity in adult offspring. Accumulation of triglycerides in non adipose tissues is commonly associated with insulin resistance. While HF diet more than doubled liver triglyceride accumulation (P< 0.0001) ((μmol/g) ADC 22.9±3.1, ADCR 5.9±0.6, ADHF 58.3±8.6 UNC 20.3±4.4, UNCR 4.8±0.3, UNHF 56.85±16.22), CR rats showed lower liver triglyceride levels (P<0.05). In skeletal muscle triglyceride content was reduced by CR (P<0.001) ((μmol/g) ADC 11.9±2.5, ADCR 1.8±0.7, ADHF 10.8±1.4 UNC 17.3±3.0, UNCR 8.2±1.4, UNHF11.8±1.0). Maternal undernutrition had no independent effect on offspring's liver or skeletal muscle triglyceride levels. Using RT-PCR we have quantified the hepatic expression of gluconeogenesis regulating enzymes PEPCK (relative PEPCK mRNA arbitrary units ADC 1.212±0.101, ADCR 2.163±0.270, ADHF 1.142±0.147 UNC 1.029±0.118, UNCR 1.771±0.130, UNHF 1.019±0.117) and pyruvate carboxylase (PC) (relative PC mRNA arbitrary units ADC 0.967±0.075, ADCR 0.620±0.042, ADHF 1.127±0.205, UNC 0.776±0.074, UNCR 0.652±0.048, UNHF 0.944±0.041). Expression of PEPCK and PC were not influenced by maternal undernutrition. Postnatal hypocaloric nutrition elevated expression of PEPCK (p<0.0001) and reduced expression of PC (P<0.05). Our data suggest that the aetiology of obesity induced by maternal undernutrition during fetal development may be a consequence of insulin hypersecretion without changes in insulin sensitivity. We therefore propose that this form of obesity involves separate pathways independent of the insulin resistant state observed in diet-induced obesity.

P2-094

Fetal Macrosomia in Women with Type-1 Diabetes Gerard HA Visser, A Kerssen, IM Evers, M Rijpert, HW de Valk. Departments of Obstetrics and Internal Medicine, University Medical Center Utrecht, The Netherlands.

Background. Fetal macrosomia (birth weight ≥ 90th centile) is a continuing and possibly increasing problem in pregnancies of women, with type-1 diabetes mellitus. In a recent nationwide study in the Netherlands, 50% of infants were found to be macrosomic at birth (BMJ 2004;328,915), despite "safe" HbA1c values (< 4SD from the mean) in 75% of women. Correlations with BMI, weight gain in pregnancy and HbA1c were poor. Currently, treatment strategies aimed at the prevention of macrosomia are focussed on the achievement of tight glycaemic control in the third trimester of pregnancy. We used a continuous glucose monitoring system (CGMS) to assess the relationship between 24h diurnal glucose profiles in all three trimesters of pregnancy and infant birth weight. **Methods**. Fifty-three pregnant women with type-1 diabetes used the CGMS during each trimester of pregnancy. The glucose profiles of the women with a normal weight infant or a macrosomic infant were compared. **Results**. Sixty percent of the women with diabetes gave birth to a macrosomic infant. Forty-one percent of these macrosomic infants were already large-for-dates on ultrasound before 30 weeks of gestation and all the latter infants had a birth weight ≥ 97.7th centile. Second trimester HbA1c was not correlated to birth weight. The mothers of the early macrosomic infants had elevated glucose levels for most of the day during the second trimester of pregnancy (p<0.05). In the third trimester, glucose variability – but not mean glucose – was higher in the women who had a macrosomic infant compared to those who had a normal weight infant (p<0.05). **Conclusions**. In women with type-1 diabetes severe fetal macrosomia of the infant starts early in pregnancy and this is likely to be caused by increased maternal glucose levels. Glucose regulation should therefore be tightened, especially during the second trimester of pregnancy. Currently we investigate the relationship between glucose control during pregnancy and birth weight with neuro-psychological development and signs of insulin resistance at 6 years of age in the 320 infants that took part in the nationwide study (grant Dutch Diabetes Foundation).

Epidemiologic / Nutrition Transition

P2-095

The Relationship Between Maternal and Cord Lipoproteins [1]Narinder Bansal, [2]Valentine Charlton-Menys, [2] Paul N. Durrington, [3]Patrick McElduff, [1]Abir Koudsi,[1]J. Kennedy Cruickshank [1] Clinical Epidemiology & [2]Cardiovascular Medicine Group, Division of Cardiovascular Sciences, University Dept of Medicine, Manchester Royal Infirmary, Manchester UK & [3]Evidence for Population Health Unit, University of Manchester, Medical School, Manchester UK

Background: There is evidence that high maternal cholesterol levels during pregnancy are associated with increased atherosclerosis during early life, but an intergenerational risk transmission of cholesterol to the foetus has not been fully explored. **Aim:** We examined the association between maternal cholesterol levels during pregnancy and that in umbilical cord blood at birth in 2 ethnic groups. **Methods:** Maternal and umbilical cord blood samples were collected at 28 weeks gestation and birth respectively from 90 healthy pregnant women of Pakistani and White European origin. Maternal plasma and cord serum total cholesterol (TC), high density lipoprotein (HDL-C), and triglycerides were measured enzymatically using a Cobas Mira analyser and low density lipoprotein (LDL-C) was calculated using the Friedewald formula. The relationship between maternal and cord cholesterol was investigated after adjustment for pre and postnatal factors collected at 28 weeks gestation and birth respectively. **Results:** Maternal TC (mean difference 0.8mmol/L; 95% CI 0.17 to 1.4, p=0.006) and LDL-C (mean difference 0.57 mmol/L; 95% CI 0.01 to 1.1, p=0.02) were significantly lower in Pakistani women than their White European counterparts, but not however in cord blood. In a multiple linear regression, cord LDL-C, HDL-C, and triglycerides showed a weak inverse but not significant association with maternal TC ($r^{2=}$ 0.12) and LDL-C ($r^{2=}$ 0.17). Maternal age was inversely associated with cord TC (β= -0.02, p=0.05) and delivery by primary caesarean was negatively associated with cord LDL-C (β= -0.28, p=0.03). In a quadratic multiple regression model, maternal total (r^2=0.19) and LDL-C (r^2=0.22) cholesterol showed a curvilinear relationship with the respective lipids in the cord. Cord TC increased with lower values of maternal TC (β= 0.61, 95% CI 0.06 to 1.16, p=0.03) and then decreased as maternal TC increased above 7mmol/L (β= -0.05, 95 % CI -.088 to -0.01, p=0.01). This relationship was also observed with cord LDL-C which showed a similar increase for lower levels of maternal LDL-C (β= 0.33, 95% CI -0.001 to 0.66, p=0.06) followed by a decrease as maternal LDL-C rose

above 4mmol/L (β= -0.05, 95% CI -0.09 to -0.005, p=0.03). As above in the linear model, maternal age was inversely associated with cord TC (β= -0.02, p=0.06) and primary caesarean with cord LDL-C (β= -0.30, p=0.02). **Conclusion:** Our data show that increasing levels of maternal TC and LDL-C (up to about 7mmol/L and 4mmol/L respectively) are associated with increased levels in the newborn and then they decline. The mechanisms for this are unclear. Ethnic differences in maternal cholesterol are not reflected in the newborn suggesting that these differences may be due to later exposure to environmental risk factors.

P2-096

Recent Increasing Tendency of Low Birth Weight Infant and Declining Average Birth Weight in Japan Hideoki Fukuoka*, Hiroko Tsukamoto*, Tatsuro Shimomura*, Megumi Haruna#, Mieko Koyasu#, Hajime Yoshihara**, Hidemi Takimoto##, Nobuo Yoshiike##, Department of Developmental Medical Sciences, the Graduate School of International Medical Sciences, the University of Tokyo, Japan, # Department of Midwifery and women's Health, the Graduate School of Health Sciences and Nursing,the University of Tokyo, Tokyo, Japan. ** Division of Obstetrics and Gynecology, Kanagawa Kyodo Hospital, Kanagawa, Japan. ## Division of Health and Nutrition Monitoring, National Institute of Health and Nutrition, Tokyo, JAPAN

Recently in Japan, the average birth weight (g) is decline from 3,194 in 1980 to 2,982 in 2003 and the rate (%) of low birth weight infant (LBW :under 2500 g) is conversely increasing from 5.1 in 1980, to 9.1 in 2002. To elucidate this trend, we compared the 866 and 628 singleton term birth records of 1992 and 2002, in Kanagawa Kyodo Hospital, Kanagawa, Japan. In this hospital, average birth weight (g) declined from 3166±379 into 3050±367 and the rate of LBW (%) increased from 3.2 to 5.6 in 1992 and 2002, respectively, showing similar general tendency during Japanese last two decades. Mothers were divided into three groups following prepregnant BMI (body weight (Kg) / square of height (M)), thin: under 18.5, normal, and obesity : over 25.0. The average birth weight (g) in each group decreased from 3016±345 to 2977±345, from 3170±357 to 3060±363 ($p<0.001$), and 3399±469 to 3183±387 ($p < 0.001$), respectively. The infant birth weight mainly depends upon the prepregnant and pregnant maternal nutrition, if she does not smoke without any placental dysfunction like preeclampsia. In Japan, the prevalence of thin women (BMI under 18.5) in their twenties are increasing from 21.0 % in 1980 to 25.1 in 2003. This hospital, however, does not any difference in prepregnant BMI, 20.9 ±4.0, 21.0±2.9, respectively. The average maternal weight gain (Kg) decreased from 10.8±3.5 to 9.5±3.7 ($p<0.01$), and maternal weight gain under 7 kg increased from 13 to 49 %, respectively. The incidence of preterm delivery is not so high. These results suggest that the Japanese declining birth weight of term is partly caused by restriction of maternal weight gain. Japanese young ladies have strong slim body desiring and dislike to weight gain much during pregnancy. So mothers should gain the optimal maternal weight during pregnancy following each prepregnant nutritional state and prohibit the fetal programming of adult diseases. Perinatal care specialists should intensely recommend of balanced and good nutrition.

P2-097

Trends in Growth Faltering, Vitamin A Deficiency and Iron Deficiency Anemia Among School-children of Northeast-Brazil Alcides da Silva Diniz, Ida Cristina Ferreira Leite, Ilma Kruze Grande de Arruda, Malaquias Batista Filho; Federal University of Pernambuco-Brazil, Mother and Child Institute of Prof. Fernando Figueira, Pernambuco-Brazil.

Background: Linear growth retardation (LGR), vitamin A deficiency (VAD) and iron deficiency anemia (IDA) are major nutritional problems in many developing countries. However the time trends of these phenomena have not been will established in most risk areas. **Aim:** to compare time trends in LGR, VAD an IDA among school-children in the last twenty years in Northeast, Brazil. **Methods:** following a two-stages sampling procedure two cross-sectional studies (1982 and 2001) were carried out involving children aged 7-11y, of both sexes, attending public schools in Recife, Northeast Brazil. VAD was assessed by serum retinol levels, IDA by Hb concentration and LGR by the anthropometric index height-for-age. **Results** The prevalences of LGR (H/A < -2 z scores), VAD (serum Retinol <0,70μMol/L) and IDA (Hb <11,5g/dL) are shown in table 1.

Table 1 – Trends in VAD, IDA and LGR among school-children, of Northeast Brazil. **1982 - 2001**

	1982		2001		
INDICATORS	%	CI*	%	CI*	status
H/A <-2 z scores	7,9	6,2 – 9,9	3,1	2,0 – 4,7	reduction
Serum retinol<0,70μMol/L	19,7	15,5 – 24,7	20,9	17,8 – 24,2	stabilization
Hb <0,70μM/L	8,8	6,0 – 12,8	18,9	16,0 – 22,0	increment

*95% confidence interval

Conclusion: The decline in the prevalence of LGR among school children is in line with a worldwide trend observed in the last years. However VAD is still prevalent and IDA increased significantly, compared to the rate found in 1982. Concerted actions towards prevention and control micronutrient deficiency are strongly recommended in this ecologic context.

P2-098

Malnutrition in Infants Co-exist with Obesity in Adults in Rural South African Communities André Oelofse[1], Serene E Schoeman[2], Mohammed A Dhansay[2], Cornelius M Smuts[2], Johanna A Laubscher[3], Carl Lombard[3], AJ Spinnler Benadé[2] [1] Centre for Nutrition, University of Pretoria, Pretoria, South Africa. [2] Nutritional Intervention Research Unit, [3]Biostatistics Unit, Medical Research Council, Tygerberg, South Africa

Background: In South Africa, as in many developing countries, the co-existence of malnutrition in the young and obesity and its associated chronic diseases in adults are observed. This problem is exacerbated by a strong shift of people to cities, hence resulting in large groups of people in nutritional transition. Inadequate health services and maternal and child caring practices are considered to be among the underlying causes of malnutrition. An integrated nutrition programme (INP), comprising health facility-based (HFB) and community-based (CB) components were introduced by government to primarily address the problem of malnutrition of vulnerable groups in South Africa. A situational analysis at selected primary health care (PHC) facilities in the Eastern Cape (EC) and KwaZulu-Natal (KZN), two provinces within South Africa, aimed to assess the existing nutritional practices as well as the nutritional status of caregivers and preschool children visiting these facilities. This would empower decision makers to develop policy, which would not only address malnutrition and its consequences, but also contribute to prevent the development of chronic diseases. **Methods**: The study had a cross sectional design and comprised of anthropometric measurements and questionnaires administered to caregivers and children (0-71 months; n=1986) visiting the primary health care (PHC) facilities in the Eastern Cape and Kwa-Zulu Natal provinces of South Africa. **Results**: For children under the age of 6 months stunting was 11% and for children aged 6 – 71 months the prevalence was 23.5%. Weight of children from households receiving food parcels were significantly lower (9.75kg vs 10.8 $p< 0.05$) when compared to children not receiving parcels. Children receiving vitamin supplements were significantly heavier and taller (11.1 kg vs 10.2kg $p< 0.05$; 80.3 cm vs 76.4cm $p< 0.05$) than those not receiving. More than 40% of caregivers were overweight or obese. Diarrhoea was reported as the leading health problem. **Conclusions**: More than 10 years after democracy has been introduced to this country, poor health care and malnutrition remains a challenge. In this population food aid seemed to have been received by the less well nourished as would be hoped. The fact that children receiving vitamin supplements seemed to have higher body weights compared to those not receiving vitamin supplements, may be due to overall better health care practices of the caregiver. In the light of the suggested association of poor fetal and infant nutrition with the development of chronic diseases in later life, the importance of addressing malnutrition in these and similar communities become imperative.

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Relationship Between Birth Weight and Handedness RJ Stratton[1], J Mulligan[2], P Betts[2], M Elia[1] [1]Institute of Human Nutrition, DOHaD Division, University of Southampton, and [2]Southampton University Hospitals NHS Trust, UK

Background: There is some controversy about the relationship between birth weight (an indicator of fetal growth, influenced by nutrition) and handedness (an indicator of brain development and function). Although some studies suggest that low birth weight is related to left handedness, others find no such relationship[1,2,3]. Such uncertainty may partly be explained by the impact of a variety of confounding factors (such as birth order, gender, smoking during pregnancy etc), which are often not taken into account. The changes in handedness over the 20th Century may also be a factor[4]. Therefore, this analysis of a prospective, longitudinal study (Wessex Growth Study, UK) aimed to evaluate the potential link between handedness and birth weight whilst controlling for confounding factors. **Methods:** The handedness of 212 children (117 boys, 95 girls; birth weight z score –0.48 SD 1.70)), with well documented antenatal and birth history (including gestational age, birth order, smoking in pregnancy, trauma during birth), was assessed at age 14-16 years, as part of the prospective Wessex Growth Study, and related to standard deviation scores (z scores) of birth weight using the 1990 UK growth charts. **Results:** Left handedness was more common in lower birth weight children (logistic regression using birth weight z scores, odds ratio (OR) 1.374 (95% confidence interval (CI) 1.105-1.709), P=0.004), and was three-fold more common in children in the lowest tertile of birth weight z score (20% left-handedness) than in the upper tertile (6%) (OR 1.83 (95% CI 1.107-3.039), P=0.019). Handedness was not significantly related to gestational age, gender, projected adult height, birth order, trauma during birth or smoking during pregnancy. Birth weight z scores remained significant in logistic regression in the presence of these confounding variables. Left handedness was more common in children with lower z scores of measured height at 16years (OR 1.526 (95% CI 1.086-2.123), P=0.015) and tended to be (but not significantly, P=0.09) more common in those with lower projected adult height (based on maternal and paternal z scores for height). The analysis was repeated by considering only the children with projected adult heights between the 20th and 80th percentile (± 1.28 z score for projected adult height; n=142). The significant relationship between left handedness and lower birth weight z score (OR 1.412 (95% CI 1.103-1.808)) and tertiles of birth weight (19% left handedness in the lowest tertile compared to 6% in the highest tertile, OR 2.171 (95% CI 1.104-4.648)) persisted in logistic regression. **Conclusion:** These results suggest that left handedness is established or 'programmed' during early development by factors that also determine or are linked to birth weight, independently of a variety of confounding factors. [1] Tan & Nettleton (1980) Cortex 16 (3) 363-373 [2] Petridou *et al* (1994) Hum Biol 66 (6) 1093-1101 [3] Powls *et al* (1996)

Dev Med Child Neurol 38 (7) 594-602 [4] McManus (2003) Right hand, left hand. Phoenix, London.

P2-100

Prevalence Of Anaemia Among Pregnant And Lactating Women In India, 1950 To 2002 G.S. Toteja and Padam Singh, Indian Council of Medical Research, Ansari Nagar, New Delhi, India

Background: Anaemia is the most prevalent nutritional disorder in the world. Anaemia affects the cognitive performance, behaviour and physical growth of infant and children. It has deteriorating effect on the immune status. Moreover, anaemia results in decreased physical capacity and work performance. The compendium prepared by the authors is an attempt to compile the information on prevalence of anaemia among pregnant and lactating women, dietary intake of iron, analysis of trends and regional variations as well as identification of gaps in knowledge and potential areas for further research. **Methods:** Information about prevalence of anaemia and dietary intake of iron from 1950 to 2002 has been collected from major studies carried out in last five decades as well as individual research papers published in various national and international journals. Information collected have been analyzed for regional level, state level and district level using simple percentage. Appropriate weights have been used for estimating the prevalence at regional levels as well as analysis of trend in last 50 years. **Results:** Approximately 165 research papers were published on prevalence of anaemia among pregnant and lactating women and around 70 research papers on dietary intake of iron by them in last five decades. Some major task force studies were also carried out on the subject. The overall prevalence of anaemia among pregnant and lactating women is around 85%. The highest prevalence of anaemia is in the eastern region (88%) of the country and the lowest in the southern region. The anaemia level was 81% during 1950-90 which slightly increased to 84% from 1991 onwards. For pregnant and lactating women the intake was 37 and 49% of RDA respectively. **Conclusion:** The existing programme of Government of India for Prevention and Control of Nutrition Anaemia for pregnant women needs to be reviewed in the light of continued high prevalence of anaemia in the group.

P2-101

Child Abuse: An Evolutionary Price for Human Development? Prof AB (Sebastian) van As, MBChb, MBA, FCS, PhD. Trauma Unit, Red Cross Children's Hospital, Department of Pediatric Surgery, Cape Town, University of Cape Town

Background Although child abuse has most probably accompanied mankind from times immemorial, it was only reported in detail as recently as 1946. The effects of childhood abuse have been well documented. Adverse childhood experiences are known to result in social, emotional and cognitive impairment, adoption of health-risk behavior and therefore lead to disease, disability and social problems and ultimately even early death. From an evolutionary perspective, child abuse and neglect can be viewed as a form of discrimination against offspring in circumstances unfavorable to parental care. The majority of severe child abuse and infanticide occurs before the age of 18 months. **Methods** We studied the differences in development and maturity between human children and primates with a particular focus on child abuse. **Results** Human children are born approximately 18 months premature when compared to chimpanzees. Human babies therefore also only acquire the mobility and independence of a newborn chimpanzee at the age of about 18 months. There are a number of factors why human infants are likely to be at higher risk for abuse and injuries as compared to other primates. These include: (1) *Extra uterine growth, (2) Extra uterine brain development, (3) Immature skeleton and growing skull and (4) Immature neurodevelopment state at birth.* Additionally, the greater dependency of the child on the mother might exhaust her and frustrate her more than mothers of more mature born primates. **Conclusion** Although Child Abuse has been extensively researched and causes are usually found to arise from a large spectrum of socio-economic factors as well as individual characteristics, it is our opinion that child abuse might result from the evolutionary process, finally shaping the human race. The extra-uterine brain development in the early years of childhood, together with the enhanced vulnerability of the child as a result of immature neuro- and motor development as well as owning an "unprotected and growing brain" seem to be an overlooked but important evolutionary reason for the "inexplicable" human child abuse.

P2-102

A Trade-off Between Early Growth Rate and Fluctuating Asymmetry in Brazilian Boys Jonathan C.K. Wells [1], Pedro C. Hallal [2], John T. Manning [3] & Cesar G. Victora [2]; [1] MRC Childhood Nutrition Research Centre, Institute of Child Health, London UK; [2] Centro de Pesquisas Epidemiologicas, Universidade Federal de Pelotas, Brasil; [3] Department of Psychology, University of Central Lancashire, UK.

Background: Discrepancies in normally symmetrical traits are assumed to result from inability of the individual to buffer environmental and genetic stresses. Fluctuating asymmetry (FA) may therefore signal the quality of an individual to potential mates, or to parents during early life. FA could signal the heritable ability to buffer stress, the "good genes" hypothesis. Alternatively, FA could signal the degree of within-lifetime exposure to stress, in particular during specific sensitive periods of development, the "good development" hypothesis. **Aims**: We tested the hypotheses that FA at 9 years of age is related to (a) fetal growth rate, (b) early infant growth rate and (c) total post-natal growth rate in boys aged 9 years from Pelotas, Brazil. **Methods**: Asymmetry was measured in all four finger digits, ear length, foot width and foot length in a sample of 172 boys. Data on weight and height were available for the time points birth, 6 months, and 9 years. These data were converted to standard deviation scores (SDS), using published UK reference data. **Results**: Fetal growth was not related to FA, however FA was positively related to total weight gain after birth ($p<0.05$). The regression model was as follows:

	Regression coefficient	Standard error	P-value
Constant	2.09	0.034	<0.001
Birthweight SDS	0.037	0.028	0.18
Change in weight SDS birth-9 yrs	0.047	0.023	0.038

This association could be broadly attributed to weight gain in the first 6 months of post-natal life ($p=0.075$). Those currently obese had significantly greater FA than those non-obese ($p<0.05$). Growth in height showed no significant associations with FA. **Conclusions**: Our results support the "good development" hypothesis, and suggest that growth rate during an early post-natal critical window, previously linked to numerous health outcomes, also has long-term effects on FA. Asymmetry may therefore act as a reliable signal of exposure to a detrimental environment in early life.

Respiratory Outcomes

P2-103

Association of Antioxidative Vitamin and Oxidative Stress Levels in Pregnancy with Respiratory Diseases during the First Year of Life Hyesook Park, Juhee Hong, Young-Ju Kim, Eun Ae Park, Kyoung Ae Kong, Hunjae Lee; Department of Preventive Medicine, Department of Obstetrics & Gynecology, Department of Pediatrics, College of Medicine, Medical Research Center, Ewha Womans University, Seoul, Korea, Department of Social Health, Inha University, In-cheon, Korea

Backgrounds: Optimal maternal nutritional status has been shown to reduce the risk of preterm birth or low birth weight, improve postnatal growth, and reduce maternal and infant morbidity and mortality. Whereas there are numerous literature relating the impacts of maternal nutritional status on subsequent birth outcome, much less is known about the long-term impacts on infant disease status after birth. Therefore, we conducted a prospective cohort study to investigate the association between maternal micronutrients status, vitamin A, C, E, folate and oxidative stress status in pregnancy and respiratory diseases and health care visits during the first year of life.
Method: We constructed Ewha Pregnant Women Cohort to investigate pregnant women who visited hospital for prenatal care during gestational weeks 24-28. After delivery, we enrolled their healthy infants and constructs prospective Ewha Infant Growth Cohort from September 2001 to April 2004. We excluded mother-and-child pairs in which the mother had experienced hypertension or diabetes during pregnancy and had multiple births for this study, which gave us 124 mother-and-child pairs for analysis. For exposure variables, we measured the levels of maternal serum antioxidant, such as vitamin A, C, E, and folate and also the levels of maternal serum oxidative stress, homocysteine and the levels of maternal urinary oxidative stress, 8-hydroxyguanosine (8-OHdG) and malondialdehyde (MDA) at 24~28 weeks of pregnancy. For dependent variables, we followed infants' postnatal health status such as respiratory diseases and hospital visits using mail questionnaires. We applied repeated-measures ANOVA with PROC MIXED to assess the significance of differences. **Results**: With regard to the disease status of infant on antioxidative vitamin, low level of folate and vitamin C were associated with increased upper respiratory disease and low level of tocopherol and retinol were with increased lower respiratory. Low level of folate, vitamin C, and retinol were associated with increased hospital visits and low level of tocopherol was related to reduced hospital visit. For the index of oxidative stress, high level of homocystein and MDA were related to increased upper respiratory disease and hospital visits. High level of 8-OHdG was related to increased lower respiratory disease. **Conclusions**: To our knowledge, the current study is the first to demonstrate the associations between maternal microvitamin and oxidative stress status and infants' respiratory diseases from birth to one year of life. From the results that maternal antioxidative microvitamin and oxidative stress level influence the disease status and health care visits in infancy, preventive strategy is needed to increase vitamin and reduce oxidative stress of pregnant women. However, there were high rate of follow up loss and various measurement errors, thus, we need to have more efforts for compete follow up and valid and reliable measurements.

P2-104

Asthma Pathogenesis: The Origin of Asthma and Its Relationship to Breastfeeding M H Shamssain; School of Health, Natural and Social Sciences, University of Sunderland, Chester Road Campus, Sunderland, SR1 3SD, United Kingdom.

Background: Asthma is chronic disease of the lungs and it is increasing in industrialised countries. The exact causes of the increasing prevalence are unknown. Most asthma develops in early childhood and environmental factors present early in life may be crucial in the development of the disease. Mother-child interface during gestation and through breastfeeding plays an important role in asthma. Environmental factors acting early in life are key determinants of the incidence of allergic disease. The development and phenotypic expression of atopic diseases depends on a complex interaction between genetic factors, environmental factors, environmental exposure to allergens and non-specific factors, such as tobacco smoke, air pollution and infection. **Methods**: We studied 7000 children, 6-7 and 13-14 year olds. We used ISAAC questionnaire (the International Study of Asthma and Allergies in Childhood). We added questions on breastfeeding, parental smoking, nutritional status of children and

mothers during infancy, and presence of allergic conditions in parents. Data were analysed by SPSS. **Results:** Children who breastfed more than 4 months showed lower prevalence rates of asthma, allergic rhinitis and atopic eczema than those who breastfed less than 4 months. Breastfed children exposed to parental smoking had lower prevalence of asthma and wheeze than those who were not breastfed. Children of atopic mothers showed a different profile of risk factors associated with atopic sensitisation compared with other children. **Conclusions:** The present study explains the potential influence of breastfeeding on asthma pathogenesis. Breastfeeding in infancy delays the onset of or actively protect children against asthma and other allergic disorders. Early childhood events had important associations with atopic sensitisation. Early developmental changes in the immune system in children might help to explain the origins and pathogenesis of asthma and thus the effectiveness or ineffectiveness of asthma therapies

Stress; Infection; Immune Function

P2-105

Immune Functions of Fetal Baboon Bone-Marrow Derived Dendritic Cells

Shanjana Awasthi and Jacqueline J. Coalson, Department of Pathology, University of Texas Health Science Center at San Antonio, San Antonio, TX-78229, USA.

Dendritic cells are the most potent antigen-presenting cells that can take up and process the pathogens or pathogens-derived ligands. After taking up the pathogens or antigens, dendritic cells move to local lymph nodes where they stimulate the antigen-specific immune response. In the present work we studied the phenotype and activation status of fetal baboon-bone-marrow derived dendritic cells (BMDCs) in response to infectious stimuli. The femoral bone-marrow samples were collected from fetal (delivered at 175 days of gestation, 95% of term) and adult baboons. The BMDCs were cultured in presence of recombinant human- GM-CSF and IL-4 for 10 days. The BMDCs were harvested on days 2, 6, and 10 and their cell-surface phenotype was studied by FACS analysis after staining with anti-human- CD11c, HLA-DP,DQ,DR, CD40, CD80 and CD86 antibodies. The phagocytic ability of BMDCs (harvested after 6 days in culture) was studied after incubating with fluorescent *Escherichia coli* bioparticles. In addition, the harvested BMDCs were also treated with *E. coli* derived lipopolysaccharides (LPS, 20□g/ml) for 24h at 37°C in 5% CO_2 atmosphere. The phagocytosis, changes in cell-surface expression of MHC class II, T cell co-stimulatory molecules and Toll-like receptors (TLR) were studied by FACS analysis. The amounts of cytokines were measured in cells-free culture supernatants of LPS-treated BMDCs by cytometric bead array assay. We found that the phenotype and morphology of fetal and adult baboon-BMDCs were similar after 2, 6 and 10 days of culture. Similar to adult baboon-BMDCs, the fetal baboon-BMDCs showed increased expression of HLA-DP, DQ, DR and TLR4 in response to *E.coli* LPS. However, the fetal baboon-BMDCs were deficient in their phagocytic ability and secreted low levels of TNF and IL-6 as compared to adult baboon-BMDCs ($p<0.05$). In conclusion, our results suggest that the fetal baboon-BMDCs are defective in mounting robust immune response against infectious stimuli. Research Support: American Lung Association

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Gender Specific Differences in Response to Changes in Glucocorticoid Concentration in the Human Fetus: Relationship to Birthweight Vicki L Clifton, Vanessa E Murphy, Peter G Gibson, Roger Smith, Warwick B Giles and Naomi Scott; Mothers and Babies Research Centre and Respiratory and Sleep Medicine of the Hunter Medical Research Institute, Newcastle, NSW, Australia

Background: We have recently reported when asthma, regardless of its severity, was not treated during pregnancy with inhaled steroids, female fetal growth was significantly reduced. The male fetus appeared unaffected by asthma or its treatment. These findings suggested mechanisms that regulate fetal growth are gender specific. Initial studies suggested that placental pathways regulated by glucocorticoids were altered in a sexually dimorphic manner. Since glucocorticoids play a central role in the growth and development of fetal organ systems, we questioned whether maternal cortisol and placental pathways regulated by cortisol differ between the male and female fetus of normal pregnancies and pregnancies complicated by asthma and whether the change in cortisol contributed to alterations in neonatal birthweight. **Methods:** Maternal plasma cortisol concentrations were measured during gestation to determine whether the presence of asthma altered hypothalamic-pituitary-adrenal function. Placental cytokine and glucocorticoid receptor (GR) mRNA expression were assessed in placentae collected at term from normal pregnancies and pregnancies complicated by asthma. Cord blood cortisol concentrations were measured and related to birthweight. Data was analysed based on sex. **Results:** Maternal cortisol concentrations were significantly increased in women with asthma (n=36) relative to non asthmatic women (n=22) throughout gestation. Cord blood cortisol concentrations were significantly higher in both male (n=44) and female (n=49) fetuses of pregnancies complicated by asthma. In normal pregnancies, female fetuses had significantly increased placental basal cytokine mRNA expression (n=10) and increased GR and MR expression (n=10, $P<0.05$) relative to placentae from male fetuses (n=10). In pregnancies complicated by asthma, female fetuses had decreased basal cytokine mRNA expression (n=10) and decreased GR and MR expression (n=10, $P<0.05$) and reduced birthweight (n=18) relative to female fetuses of the control group (n=16). Male fetuses from control (n=17) and asthmatic (n=20) pregnancies were not significantly different in any parameters measured. There was a positive correlation between birthweight centile and cord blood cortisol in male neonates (n=73, R=0.269, P=0.009) and a negative correlation in female neonates (n=71, R=0.294, P=0.017). **Conclusion:** These findings indicate that asthma is a physiological stress on the maternal system during pregnancy causing a rise in cortisol and resulting in increased cortisol in the fetal compartment. In association with that rise in cortisol, the female fetus reduces growth and down regulates pathways associated with glucocorticoid regulation. The male fetus does not respond to changes in glucocorticoid and continues to grow. These data indicate there are sex-specific differences in the human fetal response to a maternal physiological stress.

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Food Restriction During the Developmental/Newborn Period Attenuates Offspring Inflammatory Response Dave A. Gayle, Mina Desai, Ederlen Casillas , and Michael G. Ross Department of Obstetrics and Gynecology, David Geffen School of Medicine at UCLA and LABioMed at Harbor-UCLA Medical Center, Torrance, CA 90502, USA

Background: Undernourishment has long been associated with reduced immunocompetence as evidenced by epidemiological findings which show increased parasitic infections, diarrhea and pneumonia in underfed individuals. Recent experimental findings now show that offspring which were exposed to a nutrient-deprived environment during gestation and/or lactation have compromised responses to immune challenges. For example, offspring of maternal rats subjected to protein restriction during lactation demonstrate exaggerated and uncontrolled neutrophil response to infectious stimuli. Although reduced growth of the thymus and spleen partly explains these deficiencies, the impact of undernutrition during gestation and/or lactation on offspring immunocompetence is largely unexplored. This study sought to determine the effect of rat maternal food restriction during gestation and/or lactation on offspring pro-inflammatory cytokine responses to bacterial lipopolysaccharide (LPS). **Methods:** At gestation day 10, Sprague-Dawley rats were randomized to 50% food restriction (**FR**) or ad libitum (**Adlib**) access to food. At birth, all litters were culled to 8 pups (4 males and 4 females). Pups from **Adlib** dams were cross-fostered to be nursed by either ad libitum (**Adlib/Adlib** offspring) or food-restricted (**Adlib/FR** offspring) dams. Pups from food-restricted dams were cross-fostered to food restricted (**FR/FR** offspring) dams for nursing. Following lactation, all pups were given ad libitum access to regular rat chow. At 8 months of age, female offspring from each group (n= 4) were intraperitoneally administered LPS (300 μg/kg b.w.) or physiological saline and plasma was collected 6 h post-treatment via cardiac puncture. Plasma interleukin-1ß (IL-1ß), IL-6 and tumor necrosis factor-a (TNF-a) was determined by ELISA (R&D System). All values are expressed as means ± SEM. **Results:** In response to LPS, plasma levels of IL-1ß, IL-6 and TNF-a in all three groups were significantly increased ($p<0.05$) above those of saline-treated controls. For all three cytokines, levels of induction in the control (Adlib/Adlib) group was significantly ($p < 0.01$) greater than the induction in either the FR/FR or Adlib/FR groups, as follows: IL-1ß Adlib/Adlib (820 ± 32 pg/ml) > Adlib/FR (576 ± 227 pg/ml) > FR/FR (484 ± 29 pg/ml). IL-6 Adlib/Adlib (3945 ± 126 pg/ml) > FR/FR (2932 ± 681 pg/ml) > Adlib/FR (2844 ± 463 pg/ml). TNF-a levels Adlib/Adlib (204 ± 43 pg/ml) > Adlib/FR (111 ± 53 pg/ml) > FR/FR (94 ± 30 pg/ml). **Conclusions:** Maternal food restriction during gestation and lactation, or during lactation alone, decreased the adult offspring inflammatory responses to a bacterial challenge. As these pivotal cytokines activate and coordinate the inflammatory response, these results suggest that undernourishment during the developmental and newborn periods negatively affects offspring immuno-competence.

P2-108

Programming of Cardiovascular Function: Differential Effects of Pre- and Post-natal Maternal Stress N. Igosheva[1,2], P. Taylor[3], L. Poston[3], V. Glover[1] [1]Institute of Reproductive and Developmental Biology, Imperial College London [2]Saratov State University, Department of Biology, Russia [3]Maternal and Fetal Research Unit, Division of Reproductive Health, Endocrinology and Development, King's College London

Background: Environmental influences during early development are increasingly implicated in the programming of adulthood cardiovascular disorders. The majority of studies have focused on effects of maternal nutritional imbalance. We have shown recently that mild stress in the pregnant rat leads to increased cardiovascular (CV) responses to restraint stress in the offspring. The aim of this study was to further characterize the effects of maternal stress *in utero*, as well as during the suckling period, on CV function of young adult offspring. **Methods**: Sprague-Dawley female rats were exposed to restraint and bright light for 30 min thrice daily on days 15 to 21 of pregnancy. 48 h after birth all offspring were cross fostered to recently parturient control dams, and assigned to one of three groups (n=8): 1) control pups (CON) born to non-stressed dams and suckled by non-stressed dams; 2) prenatally stressed (PS) pups born to stressed dams and suckled by non-stressed dams; 3) animals born to non-stressed dams and suckled by stressed dams (SSD). At 120 days of age, CV variables were measured by remote telemetric recording at rest and during restraint stress. Reactivity of mesenteric small arteries was assessed by myography, and responses to electrical field stimulation (EFS) determined. **Results**: In adult offspring, basal systolic arterial pressure (SAP, awake phase) was elevated in female SSD (128±3 2.4 mmHg vs CON 119 ±3.08 mm Hg, by ANOVA, $P<0.05$) but not in PS offspring. Resting blood pressure in PS and SSD males was similar to CON. However, the pattern of CV responses during restraint stress and recovery differed markedly between the groups. PS rats had larger increase in SAP and DAP following restraint stress ($p<0.01$) and delayed recovery than CON ($p<0.05$). The offspring reared by stressed dams also showed higher SAP and DAP responsiveness to stress than CON ($p<0.05$). Females of

PS and SSD groups showed more delayed SAP recovery than males. In mesenteric arteries pre-activated with noradrenaline, responses to neuropeptide Y (NPY) were increased in PS offspring ($p< 0.05$) but similar to CON in SSD. PS animals also were more responsive to EFS-induced vasoconstriction than SSD ($p<0.01$) and CON ($p=0.07$). **Conclusion**s: These data suggest that maternal stress both antenatally and during suckling may lead to permanent alteration in the developing CV system. Adult female offspring reared by a stressed mother were hypertensive, whereas exposure to stress *in utero* did not affect systolic blood pressure but did induce alterations in peripheral vascular function. Increased smooth muscle sensitivity to NPY, as well as enhanced vascular responses to adrenergic nerve stimulation, may contribute to the enhanced cardiovascular reactivity seen in prenatally stressed offspring.

P2-109

Fetal Growth and the Adrenocortical Response to Psychological Stress Alexander Jones, Keith M. Godfrey, Peter Wood, Clive Osmond, Peter Goulden and David I. W. Phillips; MRC Epidemiology Resource Centre, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK.

Background: Low birthweight is associated with an increased prevalence of metabolic and cardiovascular disease. Cross-sectional studies showing that people who were small at birth have increased fasting cortisol concentrations and adrenocortical responses to pharmacological challenge have led to the suggestion that low birthweight may be associated with an increased adrenocortical response to external stressors. This hypothesis is strongly supported by experimental studies in animals which show that prenatal stress has been shown to result in the birth of offspring that exhibit abnormally fearful behaviour and have elevated neuroendocrine responses to novelty and aversive stimuli. **Aim**: To determine whether smaller but otherwise healthy term babies are more likely to demonstrate an increased glucocorticoid response to psychological stress in childhood. **Methods**: We carried out a cross-sectional study of 68 boys and 72 girls (aged 7-9 years) who have been followed since 12 weeks of gestation when their mothers took part in a study of healthy children born in Southampton, United Kingdom. Salivary cortisol profiles were obtained by the children during a restful day at home. During the study visit, a further series of cortisol measures was obtained whilst the children underwent a psychological stress test (Trier Social Stress Test for Children). Differences between rest and stress cortisol measures were used to indicate their adrenocortical stress response. **Results**: In boys, birthweight, head circumference and ponderal index at birth were strongly and inversely related to salivary cortisol responses to psychological stress ($r = -0.56$, $P < 0.001$; $r = -0.46$, $P < 0.01$ and $r = -0.50$, $P < 0.001$ respectively). These associations were independent of gestational age and potential confounding factors including obesity, social class and educational achievement. In contrast, there were no associations between size at birth and cortisol responses in girls. **Conclusions**: Our findings suggest that processes occurring during fetal life, that result in a smaller newborn, have a lasting effect on adrenocortical responses to stress in boys. Small alterations in adrenocortical activity are known to be associated with raised blood pressure, glucose intolerance, and ischaemic heart disease and long-term follow-up studies of high responders to psychological stress show that they are at increased risk of developing carotid atherosclerosis, increased left ventricular mass, and hypertension. The increased adrenocortical responses to stress that we found in boys who were small at birth may, therefore, have important consequences for their future health. Recently, it has been observed that men, who grew slowly *in utero,* are more vulnerable to the later effects of poor social and financial status on their risk of coronary heart disease. Our study suggests that prenatal adaptations of adrenocortical function in men may have a role in explaining this observation.

P2-110

Prenatal Stress Modifies Activity of the Hypothalamo-Pituitary-Adrenal (HPA) but not Open-Field Behaviour in Mature Female Guinea Pig Offspring: Interaction with the Reproductive Cycle Amita Kapoor, Stephen G. Matthews; Departments of Physiology, Obstetrics and Gynaecology and Medicine, Faculty of Medicine, University of Toronto, Toronto, Canada

Background: In humans, prenatal maternal stress has been shown to alter emotional development in childhood, however the underlying mechanism remains largely unknown. Animal studies have shown that glucocorticoids represent one of the mediators of this effect. Glucocorticoids program the fetal hypothalamic-pituitary-adrenal (HPA) axis which subsequently affects the organisms' ability to cope with stress. We hypothesized that: 1) moderate prenatal stress *in utero*, at a critical stage of fetal brain development, will affect hypothalamic-pituitary-adrenal (HPA) axis function and open field behaviour in adult female guinea pig offspring and 2) programming of HPA function and behaviour will change as a function of the reproductive cycle. **Methods:** Pregnant guinea pigs were exposed to a high frequency strobe light on gestational days 50, 51, 52 (PS). A control group was left undisturbed throughout pregnancy. Behaviour was assessed on postnatal day (pnd) 25 and 70 by measurement of ambulatory activity and wall-seeking. For the pnd70 behaviour testing, females were divided on the basis of their reproductive cycle with one group being tested during the estrous phase and another during the luteal phase. At approximately pnd80 adrenocortical activity was evaluated in female offspring by saliva sampling during various stages of the reproductive cycle. Basal salivary cortisol levels were measured on days 0, 7, 10 and 0 (estrous=day 0) of the reproductive cycle at 08:00h, 12:00h and 16:00h. Activated levels were measured on reproductive cycle days 1, 8, 11 and 1 during and after exposure (30 min) to the high frequency strobe light. **Results:** There was no effect of prenatal stress or stage of the reproductive cycle on ambulatory activity or wall-seeking behaviour at either pnd25 or pnd70. There was no effect of prenatal stress on basal salivary cortisol levels. The salivary cortisol response to the first strobe light exposure during the estrous phase of the reproductive cycle was significantly decreased in PS50 female offspring ($p<0.01$). There were no differences in the cortisol response to the strobe light during the mid and late luteal phase however, during the estrous phase of the subsequent reproductive cycle, PS50 female offspring again exhibited a significantly decreased cortisol response ($p<0.004$). **Conclusions:** Prenatal stress during critical windows of development programs activated HPA axis function in female adult offspring but does not appear to alter behaviour in a novel environment. Further, there is interaction between programming of the HPA axis and stage of the reproductive cycle. This study was supported by the Natural Sciences and Engineering Research Council.

P2-111

Adults That Have Been Small for Gestational Age at Birth Show Increased Gingival Bleeding Gunther Meinlschmidt, Olivia Bolt and Dirk H. Hellhammer; Department of Clinical and Theoretical Psychobiology, University of Trier, Johanniterufer 15, D–54290 Trier, Germany; Department of Clinical Psychology and Psychotherapy, University of Basel, Missionsstrasse 62, CH–4055 Basel, Switzerland.

Background: Previous studies have shown that low birth weight, including low birth weight for gestational age, is associated with increased risk for cardiovascular diseases in adults. However, the mediating mechanisms are largely unknown. Interestingly, animal studies have shown that manipulations early in life can lead to an increased risk for periodontal diseases. In humans, subjects with pathological oral conditions, including increased gingival bleeding, gingivitis, and periodontitis, are more likely to suffer from cardiovascular diseases. We therefore hypothesized that disruptions in oral health might represent a link between low birth weight and later cardiovascular diseases and examined whether gingival bleeding, as an indicator of periodontal health, is more prevalent in subjects with low birth weight for gestational age. **Methods:** Forty-five healthy subjects (age range: 19 – 29 years) provided complete information about their birth weight and length of gestation and examined their gingival health by evaluating their gingival bleeding on two successive mornings. The gingival bleeding was assessed by a standardized procedure, consisting of the use of dental floss and subsequent evaluation of the bleeding-degree at each tooth space with the help of a picture-based visual rating scale. Based on this information, the size for gestational age and an index for the strength of gingival bleeding were calculated. The bleeding–index data were normalized through rank–transformation. **Results:** Subjects that have been small for gestational age showed stronger gingival bleeding ($r = -.333$, $p = .026$). After controlling for potentially confounding factors, including age/year of birth and gender, as well as for potentially mediating factors, including body–mass–index, waist–to–hip–ratio, and smoked cigarettes per day, the correlation still remained significant. **Conclusions:** To our knowledge, this is the first study to show a relation between birth characteristics and gingival bleeding. Adult subjects, who were small for gestational age at birth, showed stronger gingival bleeding. It is unlikely that this relation is mediated by differences in body–mass, fat distribution, or smoking. Since periodontal disease is a risk factor for cardiovascular diseases, gingival bleeding, as an indicator of periodontal health, might be a link between low birth weight and an increased prevalence of cardiovascular diseases. Based on these findings, one may think about implications for prevention and treatment. First, as being small for gestational age seems to be related with reduced oral health, people that have been small for gestational age should take special preventive actions for maintaining their oral health. Second, treatment of oral health problems might be a way to reduce the increased risk for cardiovascular diseases in subjects that have been small for gestational age. Future studies are needed to scrutinize this relationship and the underlying mechanisms in more detail.

P2-112

Prenatal Exposure to Interleukin-6 Results in Inflammatory Neurodegeneration in Hippocampus with NMDA/$GABA_A$ Dysregulation and Impaired Spatial Learning Anne-Maj Samuelsson, Eva Jennische, Hans-Arne Hansson, and Agneta Holmäng; Cardiovascular Institute and Wallenberg Laboratory and Institute of Anatomy and Cell Biology, Göteborg University, Sahlgrenska Academy, Göteborg, Sweden.

Background*:* During pregnancy, infection or immune responses induce cytokine release, which might influence fetal neurodevelopment, leading to neurodegenerative disease in adulthood. **Methods***:* Since the hippocampus is a key area for learning and memory, we evaluated 4- and 24-wk-old rats for the effects of early and late prenatal exposure to interleukin-6 (IL-6) on hippocampal morphology, expression of mRNA for IL-6, the gamma-aminobutyric acid receptor ($GABA_A$), the NR1 subunit of the *N*-methyl-D-aspartate (NMDA) receptor, and glial fibrillary acidic protein (GFAP), caspase-3 protein and mRNA levels, and learning abilities. **Results***:* Late exposure increased serum IL-6 and hippocampal expression of IL-6 mRNA at 4 and 24 wks. All adult rats showed neuronal loss in the hilus and astrogliosis; males had losses mainly in CA2 and CA3, and females in CA1. Expression of $GABA_A$, NR1, and GFAP mRNA increased in late-exposed males and females at 4 and 24 wks. mRNA and protein levels of the apoptosis marker caspase-3 were increased in all late-exposed rats except males at 4 wks. Evaluation of hippocampus-dependent working memory in the Morris water maze at 20 wks of age showed increases in escape latency and time spent near the pool wall in all IL-6 adult rats, especially females. **Conclusion***:* These findings suggest that fetal IL-6 exposure, especially in late pregnancy and in females, leads to increased

inflammatory responses in the circulation and hippocampus, abnormalities of hippocampal structural and morphology, and decreased learning during adulthood.

POSTER SESSION III

Genetic / Epigenetic

P3-001

Maternal Protein Restriction in Microswine Induces Gene- and Tissue-specific Chromatin Changes in Kidney of Near-term Fetal Offspring Oleg Denisenko[1], Christine Abrass[1], & Susan Bagby[2]. From the [1]Department of Medicine, University of Washington, Seattle, WA 98109, and [2]Division of Nephrology, Oregon Health & Science University, Portland, OR 97239, USA.

Maternal protein/calorie malnutrition during pregnancy in animals is associated with increased risk for future development of hypertension, nephropathy and cardiovascular diseases in the offspring. Subsequent generations of offspring also have increased risk for the development of these diseases. Multiple studies have confirmed this phenomenon; however, the molecular mechanisms by which the intrauterine environment conveys future risk for disease remain to be defined. Epigenetic modification of gene expression is a strong candidate for the long-term effects of maternal diet on offspring health, as studies in animal model systems have shown that changes in DNA methylation occur. We hypothesized that maternal diet can also alter fetal chromatin, a DNA-histone complex in which histone acetylation and methylation status plays an essential role in the long-term maintenance of gene expression level. These changes, in turn, may persist to alter postnatal expression of disease-related genes in offspring. To test this hypothesis, we used microswine model of intrauterine growth restriction (IUGR) induced by maternal low protein diet. Pregnant animals were fed ab libitum normal (NP) or protein restricted (LP) diets during the last trimester of pregnancy and first 2 wks postnatally. Offspring were examined two days prior to term (near-term), and as young adults. Adult LP offspring, as compared to normal animals, were hypertensive and had increased kidney levels of Angiotensin II. To determine if these changes were programmed in utero, we analyzed near-term fetal tissues and found by Western blot analysis that the global level of methylation of histone H3 lysine 4 was lower in fetal kidney cortices in LP than in NP animals. Next, we found that Angiotensinogen (Aogn) expression was higher in fetal kidney cortices of LP than of NP animals. Consistent with this observation, chromatin immuno-precipitation (ChIP) assays revealed changes in methylation of histone H3 Lysines 9 and 27 at the Aogn locus in LP kidney cortices. Methylation of these residues was not changed at the control GAPDH gene locus; nor was it changed in livers from the same animals. This indicates that maternal protein restriction triggers gene- and tissue-specific chromatin changes in near-term fetal offspring. Because histone methylation is not easily reversible, these changes may persist to adulthood and contribute to the long-term effects of IUGR on gene expression and thus on cardiovascular disease risk.

P3-002

Genetic and Environmental Influence on Birth Weight and Birth Length Determined by Studies in Twins Louise Grunnet[1], Signe Vielwerth[1,2], Allan Vaag[1] & Pernille Poulsen[1]. [1]Steno Diabetes Center, Gentofte, Denmark, [2] Department of Neonatology, Rigshospitalet, Copenhagen, Denmark

Background and aims: Birth weight is associated with the development of several diseases in adulthood including insulin resistance and type 2 diabetes. This association may be due to a common genotype resulting in both low birth weight and metabolic abnormalities or be a result of a prenatal environmental insult. Using a classical twin design, the aim of this study was to determine the extent to which birth weight and birth length is determined by genetic versus non-genetic factors. **Subjects and methods:** Two twin cohorts were investigated - cohort A included 110 young (aged 22 – 31 years) and 86 elderly (aged 57 – 66 years), same sex, monozygotic (MZ) and dizygotic (DZ) twins without known diabetes. Cohort B was population-based and included 606 elderly, same sex, MZ and DZ twins aged 54 – 74 years. Birth weight and length were obtained from original midwife records. Biometric modelling based on linear structural equations was used to determine the relative impact of additive genetic (A), shared environmental (C) and unique environmental (E) effects. **Results:** In the young twins in cohort A, birth weight fitted an ACE model (a^2= 0.54, c^2=0.20, e^2=0.26) indicating a major genetic influence. In contrast, birth weight data in elderly twins from cohort A fitted a CE model (c^2=0.58, e^2=0.42) including shared and unique environmental components. This finding was confirmed in the population-based cohort of elderly twins (cohort B) with an ACE model (a^2=0.37, c^2= 0.38, e^2= 0.25) as best fitting model, proposing only a minor genetic influence on birth weight. In both young and elderly twins (from cohort A & B), the best fitting model for birth length was a CE model, comprising only environmental components. **Conclusion:** The relative contribution of genes versus environment on birth weight exhibited a time (or cohort) dependency with a greater relative impact of genes among young as compared to elderly twins. Birth length, on the other hand, seemed to be exclusively influenced by non-genetic factors in both young and elderly twins.

P3-003

The Fetal Insulin Hypothesis: Offspring Birthweight is not Inversely Associated with Paternal Insulin Resistance. Knight B[1,2], Shields B[1], Hopper H[1,2], Hill A[1,2], Powell R[3], Hattersley AT[1]: 1. Exeter Family Study of Childhood Health, Peninsula Medical School, Exeter, UK. 2. Maternity Unit, RD&E Hospital, Exeter, UK. 3. Research and Development Support Unit, Exeter, UK.

Background. The association between low birth weight and diabetes, hypertension, and vascular disease in later life is well established, but the underlying mechanism is unclear. Barker and colleagues suggests that fetal malnutrition in utero, results in reduced fetal growth, and "programming" of the fetus to be insulin resistant An alternative hypothesis "the fetal insulin hypothesis" is that low birth weight and Type 2 diabetes are two phenotypes of the same genetic predisposition to beta-cell dysfunction and/or insulin resistance This genetic predisposition results in reduced insulin-dependent fetal growth in utero, hence low birth weight, as well as predisposing to adult disease. **Methods**. The Exeter Family Study of Childhood Health (EFSOCH) was established to prospectively test the Fetal Insulin Hypothesis. This is a community based study within central Exeter (UK). Parental fasting biochemistry, and anthropometry were measured at 28 weeks gestation, and offspring birthweight recorded on 990 subjects, with singleton, non diabetic pregnancies. We tested if there was an inverse association between measures of paternal insulin resistance, and offspring birthweight as predicted by the fetal insulin hypothesis. Ethical approval was given by the North and East Devon local ethics committee. **Results.** Offspring birthweight was not significantly correlated with paternal insulin resistance(r=-0.012, p=0.714), HDL cholesterol (r=-0.008, p= 0.808), or triglyceride (r=0.016, p= 0.683) when corrected for paternal BMI, and common confounders. Multiple linear regression analysis confirmed that paternal insulin resistance was not an independent predictor of offspring birthweight. **Conclusion.** Our results suggest that in a young adult Caucasian population, with non-diabetic, singleton pregnancies, offspring birth weight is not inversely associated with paternal insulin resistance.

P3-004

Recombinant Erythropoietin Administration after Cerebral Hypoxia - Ischemia Affects Histone H3 Acetylation and Methylation 30 days Post Insult Robert H. Lane, Ronald J. McPherson, Xingrao Ke, Robert A. Mcknight, Sandra E. Juul. Division of Neonatology, University of Utah School of Medicine, Salt Lake City UT; Division of Neonatology, University of Washington, Seattle, WA USA

Background: Recombinant erythropoietin (rEpo) is neuroprotective in models of neonatal brain injury. Our laboratory group has used unilateral carotid artery ligation followed by oxidative stress in the neonatal mouse to study cerebral hypoxia – ischemia, and we have determined that rEpo administration at the time of acute injury improves cerebral histology and behavioral measures up to two months after injury. Both RNA and protein expression are required for such neuroprotection. The molecular mechanism though which rEpo induces these changes in gene expression and the long term improvement in phenotype are unknown. Interestingly, studies in hemopoietic tissues demonstrate that rEpo affects histone modifications. **Hypothesis:** We hypothesized that rEpo administration affects histone acetylation and methylation in brain ipsilateral of the ligation versus vehicle treatment. **Methods:** The right carotid arteries of anesthetized 10 day old BALB-c mice were divided by electrocautery. After a 2 hour recovery period, animals were exposed to hypoxia (8% oxygen x 15 minutes) alternating with hyperoxia (100% oxygen x 10 minutes) for a total of 45 minutes of hypoxia plus 20 minutes of hyperoxia at 34°C. Sham animals underwent anesthesia and visualization of the carotid artery, but no ligation and no exposure to hypoxia – hyperoxia (n = 4). After injury, mice were treated with either rEpo (5,000 U/kg/x3 q 12) (n = 3) or vehicle control (n = 3). 30 days after injury, brains were evaluated. Histone modifications were quantified using Western blotting, with total histone as an internal control. **Results**: Results are expressed as percent of right sided vehicle control (100%). In general, rEpo administration increased H3 lysine 14 acetylation, as well as H3 lysine 4 and lysine 9 trimethylation, on the right side of the brain (injured) versus vehicle treated controls. Interestingly, modifications associated with transcription were generally increased on the left side of the brain versus the right.

Histone Modification	R Epo	R Sham	L Vehicle	L Epo	L Sham
Histone H3 Lysine14 (H3/K14) Acetylation	220 ± 30%*	121 ± 12%	648 ± 248%	586 ± 120%*	580 ± 60% **
H3/K9 Trimethylation	272 ± 18%***	142 ± 14%*	119 ± 30%	95 ± 9%	103 ± 6%
H3/K9Acetylation	92 ± 6%	91 ± 7%	203 ± 25%*	114 ± 13%	166 ± 7%
H3/K4 Trimethylation	241 ± 30%**	127 ± 13%	658 ± 70%**	780 ± 160%*	878 ± 190%*

(*p < 0.05; **p < 0.01; ***p < 0.005)

Conclusion: We conclude that brains exposed to hypoxia – ischemia and treated with rEpo n the neonatal period are characterized by altered chromatin structure when compared to brains treated with vehicle only. Because of the significant role that chromatin plays in regulating gene expression and subsequent phenotype, our findings suggest that the rEpo-treated brains are phenotypically different from vehicle-treated controls, which is consistent with our previous findings of histological and functional improvement in these animals.

P3-005

Paraoxonase Polymorphism and Vitamin Levels During Pregnancy Affect Maternal Oxidative Stress and Neonatal Birthweights Hyesook Park[a], Jungwon Min[a], Bohyun Park[a], Young Ju Kim[b], Junghyun Park[d], Hwayoung Lee[c], Eunhee Ha[a], Yun Chul Hong[e] [a]Department of Preventive Medicine, [b]Department of Obstetrics and Gynecology, [c]Department of Anatomy, [d]Medical Research Institute, Ewha Womans University, and [e]Department of Preventive Medicine, Seoul National University, Seoul, South Korea

Background The aim of this study was to determine the association between maternal oxidative stress and adverse birth outcome, and serum vitamin levels and paraoxonase (PON) polymorphism during pregnancy. **Methods** We investigated 276 pregnant women who visited a hospital for prenatal care during gestational weeks 24 and 28. We measured serum vitamin C and E levels and urinary levels of 8-hydroxy-2-deoxyguanosine (8-OH-dG). We determined the presence of a maternal PON polymorphism (*Q*-to-*R* substitution at a nucleotide located on 7q21.3) using a polymerase chain reaction-restriction fragment length polymorphism assay. Data analysis was carried out using the statistical package SAS (version 8.0). **Results** Firstly, the levels of oxidative stress were significantly different between PON1 genotypes ($p<0.05$); the *R/R* genotype had particularly high values of 8-OH-dG. Secondly, the value of 8-OH-dG was significantly lower, gestational age higher, and birthweight higher in the high- compared with the low-vitamin-E-level group. Thirdly, the *Q/Q* and *Q/R* variants with the high vitamin C and E levels exhibited reduced 8-OH-dG levels compared with those with the low vitamin C and E levels, the gestational age was higher and a similar tendency was shown for birthweight. **Conclusions** We evaluated the protective effect of combined vitamin C and E treatment during pregnancy and PON1 gene polymorphisms on the adverse effects of 8-OH-dG on birth outcome. Our result suggests that combined higher vitamin C and E levels were influenced by PON1 genotype polymorphisms, having particularly positive effects on birth outcome and reducing oxidative stress in the *Q/Q* variants with high vitamin C and E levels than in the other subjects. However, high levels of vitamins C and E still have a protective effect on gestational age and birthweight even in pregnant women with the high-risk *R/R*-type PON1 genetic polymorphism.

P3-006

Epigenetic Regulation of Gene Expression in ß-cells of Growth Retarded Rats Rebecca A Simmons, Jun Park, Irina Suponitsky-Kroyer. Department Pediatrics, University of Pennsylvania School of Medicine, Children's Hosp. Philadelphia, Philadelphia, PA, United States.

IUGR has been linked to later development of type-2 diabetes. Our model of intrauterine growth retardation in rats results in reduced ß-cell mass and onset of diabetes in adulthood. Transcription of Pdx-1, a critical ß-cell homeobox transcription factor, is decreased in fetal IUGR rats. Reduction in Pdx-1 results in decreased ß-cell mass. Suppression of Pdx-1 expression persists after birth, implicating an epigenetic mechanism. ROS are increased in IUGR islets and Pdx-1 expression is decreased in ROS treated INS-1 cells (β-cell line), suggesting that oxidative stress may underlie reduced Pdx-1 in IUGRs. The proximal promoter of Pdx-1 contains a highly conserved USF-1 binding site that is obligate for Pdx-1 transcription. The aims of this study were to determine (1) if IUGR and (2) oxidative stress impairs USF-1 binding which in turn induces histone modifications, followed by DNA methylation, thereby locking in suppression of Pdx-1 transcription. Bilateral uterine artery ligation (n=12 rats) was performed on d19 gestation (term=22d). Controls were sham-operated animals (n=12). Islets were isolated from 1 and 7 week-old rats. INS-1 cells were cultured for 3 or 24h in rotenone (increases ROS production). Chromatin was immunoprecipitated from islets or cells using antibodies to USF-1, acetyl-H3-lys9, dimethyl-H3-Lys4, or dimethyl-H3-Lys9. A 221 bp sequence of the proximal Pdx-1 promoter, incorporating the USF-1 binding site, was amplified and PCR products were separated and quantified. USF-1 binding was markedly reduced in 1 and 7 week-old IUGR islets (50% and 20% of controls, 1 and 7 weeks, respectively) ($p< 0.05$). This was associated with blunted histone acetylation at Lys9 and histone dimethylation at Lys4, markers of chromatin silencing. Both MeCP2 (a methylated DNA binding protein) and Dnmt1 (a DNA methyltransferase) binding were increased in IUGR islets suggesting that these two proteins may interact to link DNA methylation and chromatin remodeling. Rotenone treatment (24h) of INS-1 cells decreased USF-1 binding, acetylation at H3-Lys9 and dimethylation at H3-Lys4. 24h rotenone treatment additionally increased dimethylation at H3-Lys9 (all $p<0.05$ vs control). Our results show that IUGR via oxidative stress induces chromatin remodeling which acts to repress transcription of Pdx-1. This suggests that epigenetic programming can be induced by an adverse intrauterine milieu and may be one mechanism underlying fetal programming.

P3-007

Fetal Arterial Hypertension in Growth Retardation Syndrome Pavel B.Tsyvian , Tatiana V.Markova, Svetlana V. Mikhailova ; Institute of Immunology and Physiology, Russian Academy of Sciences,Mother and Child Research Institute Yekaterinburg, 620028, Russia

Background. Intrauterine growth retardation (IUGR) complicates up to 10% of all pregnancies. Although perinatal complications of IUGR are well documented, only recently the researchers are focused on the long-term morbidity that is associated with this phenomenon. Numerous epidemiological studies have suggested that IUGR is a risk factor for the development of the essential hypertension in the later life. Earlier, we have demonstrated that fetal left ventricular (LV) isovolumic relaxation time (IRT) is significantly longer in IUGR than in normally developing fetus (Tsyvian e.a. Ultrasound in Med.Biol.1995, 21,739-743). Strong relationship between IRT and end-systolic ventricular pressure (arterial pressure) was shown in lambs (Leeuwenburgh e.a. Am.J.Physiol. 2002, 282(4),H1350-8) which makes possible noninvasive assessment of blood pressure through ultrasound measurement of IRT. Our hypothesis is that ultrasound evaluation, through the measurement of fetal LV IRT, may be used to identify at-risk infants who are likely to experience hypertensive disease later in life. *The objective* of our study was to determine whether ultrasonically obtained in utero measurements of LV-IRT and postnatal measurements of rennin and angiotensin I concentrations in the umbilical blood, obtained after delivery, differ between normally developing fetuses and fetuses with IUGR. **Methods**.A total 38 women with pregnancy duration varied between 32 and 40 (median 36) weeks consented to participate in the study. Pregnancy was uneventful in 22 women. IUGR, as expressed by an upper-abdominal circumference below 5th percentile and umbilical artery pulsatility index above 90th percentile was diagnosed in remaining 16 pregnancies. IUGR was induced by preeclampsia. LV IRT (ms) was determined using combined two-dimensional, pulsed Doppler recording system (Aloka SSD 1400, Aloka Industry Corporation, Tokyo). The Doppler sample volume was placed immediately distal to the mitral leaflets. On the Doppler waveform traces IRT was determined from the artefact of aortic valve closure to the onset of transmitral flow. Renin and angiotensin I concentrations were determined by radio-immuno assay method in the 3 mL samples of umbilical blood. Arterial systolic blood pressure (BP) was measured at 1st and 5th days of life. **Results**. In IUGR fetuses, mean LV IRT 62±8 ms was longer ($p=0.03$) than in appropriate for gestational age (AGA) fetuses 47±6 ms. Mean birth weight in IUGR newborns was 2720±214g, in AGA newborns the mean weight was 3252±162g. ($p<0.01$). Active plasma renin concentration (7.78±1.03 ng/ml) and angiotensin I concentration (4.21±0.70 ng/ml) in IUGR newborns were significantly more than in AGA group. In AGA newborns renin concentration was (4.81±1.04 ng/ml, $p<0.001$), angiotensin I concentration was 2.69±0.44 ng/ml, $p<0.001$). There was no significant difference in systolic BP between groups at 1st day of life: 46±4 mmHg in IUGR and 52±6 mmHg in AGA. At 5th day BP in IUGR group was 76±5 vs 60±6 mmHg ($p< 0.01$) in AGA group. **Conclusions**. Both chronic hypoxia and decreased renal perfusion stimulate renin synthesis in IUGR (Robillard e.a. Am.J.Physiol.1988, 254, F771-9). The endpoint of the stimulation of renin-angiotensin cascade is the vasoconstriction and positive inotropic effect of angiotensin II on the fetal myocardium which results in hypertension. We propose that left ventricular isovolumic relaxation time could be a sensitive index of fetal hypertension in growth retarded fetus. Further studies needed to evaluate the role of fetal hypertension in the development of hypertensive disease in later life.

Infant Feeding & Postnatal Growth

P3-008

Growth Patterns in Adolescent Risk Groups Defined by Lipid Profiles, Insulin Resistance and Blood Pressure Linda Adair, Thomas McDade, and Christopher Kuzawa. Department of Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 USA, Department of Anthropology, Northwestern University, Evanston, IL.

Background. Most studies of developmental origins of cardiovascular disease (CVD) examine single risk factors or outcomes, ignoring the common clustering of risk factors. Our objective was to identify the birth characteristics and growth trajectories of youth clustered into sex-specific groups according to their plasma lipids, blood pressure (BP), fasting glucose and HOMA insulin resistance. **Methods**: We use data from the ongoing Cebu (Philippines) Longitudinal Health and Nutrition Survey (CLHNS), a community based study of a 1983-84 cohort of 3,080 singleton births. Mothers were recruited and assessed during pregnancy, then child weight and length a were measured every 2 months from birth to 24 months. Further anthropometric assessments took place at 8.5, 11.5, 14-15, and 19 years of age. The present analysis includes 599 youth who were part of a CVD risk substudy conducted in 1998. The substudy sample included all youth who were full term and weighed <2.6 kg at birth (n=133), and a random sample of youth who were full term and weighed =2.6 kg. Fasting triglycerides, HDL-cholesterol, LDL-cholesterol, total cholesterol to HDLc ratio, glucose, HOMA calculated from glucose and insulin, and systolic and diastolic BP were transformed into sex-specific Z-scores before being entered into a kmeans cluster analysis. The cluster analysis produced three groups with common characteristics in boys and girls. A "low risk" group (n=133 girls, 124 boys) had negative Z-scores for all risk factors except HDLc. Two high risk groups were identified. One (n=98 girls, 98 boys) had elevated BP but no adverse lipid values or evidence of insulin resistance (IR). The third group (n=71girls, 74 boys) had elevated fasting glucose and HOMA and adverse lipid values. Multinomial logistic regression was used to identify significant predictors of being in one of the 2 higher risk clusters compared to the low risk cluster, controlling for socioeconomic status (a multidimensional index of income, assets and maternal education) at birth and in adolescence. Growth curves were plotted separately for males and females in each group to examine trends in linear growth and BMI from birth, and skinfold thicknesses from age 8 onward.

Results: Despite having a low prevalence of overweight (2.3% defined by IOTF BMI cutpoints), more than half of the adolescents had HDLc<40 mg/dl; 19.8% of girls, and 11% of boys had LDL>130 mg/dl. Among girls, birth weight<2.6 kg doubled the odds of being in the adverse lipids-insulin resistance group (OR 2.11, 95%CI 1.05-4.26), but birth weight was not associated with risk group membership in boys. Growth patterns in the 3 groups began to diverge in infancy. While there were no differences in linear growth, weight, and thus BMI was consistently higher in the high BP and adverse lipid-IR groups after 6 months of age. **Conclusion**: These results suggest an important role for early life influences on the development of CVD risk, of particular importance in this relatively lean population with a low dietary fat intake.

P3-009

Size at Birth and Growth Trajectories to Young Adulthood Linda Adair, Department of Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 USA

Background: Few studies in developing countries follow growth trajectories from birth to adulthood. Such studies are important because size at birth *and* postnatal growth relate to risk of chronic disease in adulthood. **Objective**: This study examines the interrelationships of maternal factors during pregnancy, infant birth weight and length, early postnatal growth, and young adult height, weight, BMI, and skinfold thicknesses, with particular attention to patterns of growth associated with increased chronic disease risk. **Methods**: Women were recruited in pregnancy, and offspring were followed from birth to age 19 in the community-based Cebu (Philippines) Longitudinal Health and Nutrition Survey. The analysis sample includes all young adult males, (n=1040), and non-pregnant females (n=871) measured at age 19 who had height and weight data at birth and age 2 yr. Associations were assessed in a series of sex-stratified models in which various measures of adult size were regressed on birth weight or length. Preterm birth, birth order (firstborn or not), maternal height and arm fat area during pregnancy, maturation status, SES at birth and SES slope from birth to adulthood were assessed individually as potential confounders, mediators, or modifiers of the relationship of birth size to adult size. The relative importance of birth size and size at 2 years of age as predictors of adult height and BMI was assessed in models which included birth weight or length z-score a residual calculated from sex-stratified regression of length, or BMI at age 2 on birth weight or length, depending on the models being tested. Additional analyses examined growth trajectories of individuals who were relatively thin at birth (lowest third of the BMI distribution), but relatively heavy as adolescents (highest third of the BMI distribution *Results*: Birth weight and length are independently, positively associated with height, BMI and sum of skinfolds at age 19 males and females, and inversely associated with the subscapular to triceps skinfold ratio in males only. The effects of size at birth on adult size were modified by birth order, and remained significant after adjusting for maternal nutritional status, socioeconomic status at birth and throughout the growth period, and maturation. Early postnatal growth was strongly influenced by BMI at birth, with rapid early infant weight gain associated with thinness. The growth pattern of the at-risk group most often associated with increased risk of chronic disease (small at birth, relatively heavy as an adult), was characterized by more rapid growth in the first 4 postnatal months. **Conclusions**: The high level of interrelatedness of maternal nutrition in pregnancy, prenatal growth and postnatal growth emphasizes the need to consider the full growth trajectory in studies of developmental origins of adult disease.

P3-010

Circulating Leptin Levels and Postnatal Growth in Two Generations of Sprague-Dawley Rat Offspring Exposed to Reduced Fetal Perfusion: Cindy M. Anderson; College of Nursing, University of North Dakota, Grand Forks, North Dakota, USA

Background: Fetal malnutrition, in the form of reduced perfusion during a critical period of development, combined with exposure to reduced maternal leptin levels during late gestation, may contribute to the link between the adverse fetal environment and alterations in growth, triggering the development of adult onset disease. Pregnancy is associated with increased circulating leptin levels, possibly serving as a fetal growth factor. Recently, our lab reported that pregnant dams with reduced utero-placental perfusion pressure had significantly lower circulating levels of plasma leptin and evidence of fetal growth restriction, compared to control dams. Further, we identified the development of hypertension and mesenteric artery hyperresponsiveness in F1 offspring exposed to reduced utero-placental perfusion pressure, perpetuated in their F2 progeny. During late gestation, maternal leptin levels have been correlated with fetal leptin levels, suggesting the potential for altered fetal growth in a low leptin environment. It is unknown whether exposure to low maternal leptin levels leads to altered circulating leptin levels in offspring later in life. Thus, this study was designed to determine the influence of reduced utero-placental perfusion during late gestation on circulating leptin levels and postnatal growth, and perpetuation of these effects in F2 offspring. **Methods**: Experimental dams (F0) underwent a surgical procedure to reduce utero-placental perfusion pressure (RUPP), with resulting offspring comprising the first generation (F1). Offspring of control dams served as the control group (C). One male and female from each of the F1 experimental litters were randomly selected as breeders of the second generation (F2). Offspring were identified and weighed within 12 hours of birth, with weights obtained weekly thereafter through 12 weeks of age for F2, and through 24 weeks of age for control and F1. Organ weights and plasma leptin levels were determined in all groups at 6, 9 and 12 weeks. **Results**: Birth weight was significantly reduced in F1 male offspring (5.93 $\pm$ 0.11g, n=52; $p < 0.01$) compared to control (6.33 $\pm$ 0.06g, n=89) while F2 male birth weight (6.73 $\pm$ 0.08g, n=30) was significantly increased over both control ($p < 0.05$) and F1 ($p<0.001$). Among females, F2 offspring weighed significantly more at birth (6.34 $\pm$ 0.05g, n=37), compared to control (6.0 $\pm$ 0.07g, n=73; $p < 0.05$) and F1 (5.92 $\pm$ 0.08g, n=61; $p<0.01$). There were no significant differences in birth weight between control and F1 female offspring. At 3 (F1, 46.25 $\pm$ 1.55g vs. C 43.73 $\pm$ 0.96g), 6 (F1, 180 $\pm$ 3.91g vs. C, 170.1 $\pm$ 2.43g) and 12 weeks (F1, 400.4 $\pm$ 12.27g, n=27 vs C, 370 $\pm$ 6.89g, n=53), F1 males weighed significantly more than control ($p< 0.05$). F1 female offspring weighed significantly more than control at 3 weeks (48.77 $\pm$ 1.33g, vs 43.64 $\pm$ 1.1g respectively, $p < 0.01$), with no further significant differences at any other time points. Organ/body weight ratios (heart, liver, kidney) demonstrated a pattern similar to postnatal growth. Leptin levels were increased in F1 females at 12 weeks (3.32 $\pm$ 0.64ng/ml, n=6) compared to control (2.7 $\pm$ 0.35ng/ml, n=6; $p < 0.05$) and F2 (1.44 $\pm$ 0.14ng/ml, n=5; $p < 0.001$), and in F1 males at 9 weeks (5.86 $\pm$ 1.19ng/ml, n=7) vs. F2 (2.73 $\pm$ 0.2ng/ml, n=5; $p < 0.05$). A pattern of increased leptin levels in males vs. females within groups was also apparent. **Conclusions**: These findings indicate that reduced in-utero perfusion leads to gender differences in growth patterns and circulating leptin levels. While birth weight was significantly decreased in F1 male offspring, enhanced growth characterized F1 postnatal growth from 3-12 weeks, with leptin levels following a similar trend. Differences in female postnatal growth were evident at weaning, with a trend of increased weight in F1 females. These findings suggest fetal exposure to decreased maternal leptin levels may alter postnatal leptin levels and growth in offspring.

P3-011

Growth Rate during Early Life is Associated with Body Mass Index and Fat Mass at Pre-pubertal Age in Very Low Birth Weight Premature Children Dawei Wang, Stephanie A. Atkinson, Sue Steele, Jovana Kaludjerovic; Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

Background: Rapid early growth in infants may be an important determinant of excess adiposity in childhood and adolescence owing to metabolic programming. Since current nutritional management of premature infants includes aggressive nutrition in early life to promote "catch-up" growth, such infants may be at particular risk of developing overweight and obesity in childhood. **Objective:** To examine the association of velocity of weight growth from birth to one year corrected age (CA) in VLBW infants with body mass index (BMI), total body fat (TBF), fat mass index (FMI) and abdominal visceral adipose tissue (VAT) at pre-pubertal age. **Methods:** Former VLBW premature infants (43 males/38 females; mean birth weight: 892±259 g; mean gestational age: 27.6±2.6 wk) previously enrolled in neonatal nutrition studies were followed to assess growth at pre-puberty (age range: 5-10 yr). Postnatal weight growth rate was recorded from birth to term, term to 3 m, 3 m to 6 m, 6 m to 1 year CA. BMI and BMI %ile at pre-puberty were calculated from www.bcm.tmc.edu/bodycomlab/ based on subjects' current body weight and height. TBF and regional analysis of visceral adipose tissue were measured by dual energy x-ray absorptiometry (Hologic QDR 1000W). FMI was calculated as fat mass/height ^{2}. **Results:** Data (mean±SD) are summarized in Table.

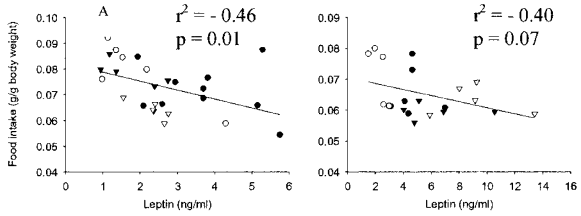
Variables	Males	Females
BMI (kg/m^2) at pre-puberty	15±2	16±2
BMI %ile at pre-puberty	34±32	40±29
%TBF at pre-puberty	18.5±4.4	20.9±3.3
FMI (kg/m^2) at pre-puberty	2.8±1.1	3.2±0.9
Total regional VAT (g)	254±242	352±311
VAT/TBF (%)	47±13	40±6
? Weight (birth to term, g/wk)	139±32	133±32
? Weight (term to 3 m, g/m)	831±351	672±165
? Weight (3 m to 6 m, g/m)	510±163	481±121
? Weight (6 m to 1 year, g/m)	321±95	312±58

For preterm males, a significant association was observed between BMI (r=0.32, p=0.047, n=38) and BMI %ile (r=0.38, p=0.02, n=37) at pre-pubertal age with growth rate from birth to term CA. For preterm females, a significant associated was observed between %TBF (r=0.43, p=0.03, n=26) and FMI (r=0.40, p=0.04, n=26) values at pre-pubertal age with growth rate from 6 m to 1 year CA. VAT representing central adiposity was not correlated with early growth velocity. **Conclusions:** A higher velocity of weight growth from birth to term or up to 1 yr CA may be a determinant of higher BMI and/or body fat mass at pre-puberty for former premature infants. Thus, rapid "catch-up" growth in early life may have a negative impact on body composition in childhood and potentially later health outcomes.

P3-012

A Low Protein Maternal Diet During Pregnancy and Lactation has Sex and Window of Exposure Specific Effects on Offspring Serum Leptin and Growth in the Rat Claudia Bautista[1], Fernando Larrea[1], Peter Nathanielsz[2], Elena Zambrano[1]. [1]*Department of Reproductive Biology, Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubirán, Mexico City, Mexico;* [2]*Center for Pregnancy and Newborn Research, University of Texas Health Sciences Center San Antonio, TX, USA*

Background. Several studies report an increased incidence of obesity in adulthood following undernutrition during the early stages of development, a relationship that has been termed metabolic programming, which appears to be mediated in part, by changes in leptin action. We determined the effects of protein restriction during pregnancy and/or lactation on growth, food intake and serum leptin in male and female offspring at 100 days post natal life. **Methods.** We fed Wistar rats a normal control 20% casein diet (C) or a restricted diet (R) of 10% casein during pregnancy. After delivery, mothers received C or R diet during lactation to provide four offspring groups CC (first letter maternal pregnancy diet; second maternal lactation diet), RR, CR, and RC. After weaning offspring ate C diet *ad libitum*.. Body weight and food intake were recorded daily; serum leptin determined by RIA at 100 d of age. Data are presented as mean ± SEM from 5 to 6 litters per group. Differences between groups compared using ANOVA. Leptin and food intake (g/g body wt) were correlated by Pearson correlation. p=0.05. **Results.** Female but not male R pups weighed less than C at birth. Serum leptin was reduced in RR and CR female pups compared with CC and increased in RC males compared with CC at 100 days of age (Table 1). Body weight at 100 days was reduced in RR and CR female and male pups. When food intake is expressed per unit body weight, there is a negative correlation in the female pups, food intake decreases while leptin increases (Fig 1A). The same tendency is observed in male pups (p=0.07). There is a positive



correlation between leptin and body weight in the female pups. There is also a positive correlation between body weight and absolute food intake in both, male and female pups. **Conclusion.** Maternal protein restriction in the rat alters serum leptin concentrations and growth in the female and male offspring in a gender and time window of exposure specific manner.

Fig. 1. A Female and **B** Male pup correlations in all 4 groups between leptin and relative food intake.
CC ( ); RR ( ); CR ( ); RC ( ). For the pairs with negative correlation, one variable tends to decrease while the other increases. For pairs with p values greater than 0.05, there is no significant relationship between the two variables.

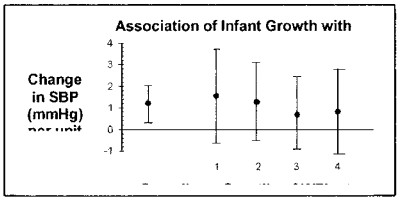
Table 1 Female and Male pup data at 100 days of age. Maternal diet denoted as explained in text. Mean ± SEM, p = 0.05 for data between groups not sharing at least one letter.

Group	Sex	Body weight (g)	Food intake (g)	Leptin (ng/ml)
CC	F	319.2 ± 9.9a	22.8 ± 1.03a	3.4 ± 0.46a
RR	F	248.7 ± 16.2b	19.4 ± 0.19b	1.4 ± 0.21b
CR	F	257.7 ± 10.4b,c	19.5 ± 0.42b	1.8 ± 0.30b,c
RC	F	311.0 ± 25.1a,c	20.1 ± 2.21a,b	2.3 ± 0.28a,c
CC	M	513.2 ± 9.4a	33.7 ± 1.77a	4.7 ± 0.83a
RR	M	417.4 ± 10.8b	29.8 ± 1.01a,b	2.3 ± 0.26a
CR	M	446.3 ± 14.0b	26.6 ± 0.76b	5.2 ± 0.59a
RC	M	517.2 ± 9.7a	32.5 ± 0.49a	9.3 ± 1.56b

P3-013

Size at Birth, Infant Growth, and Blood Pressure at Months of Age Mandy B. Belfort[1,2], Sheryl L. Rifas-Shiman[1], Janet Rich-Edwards[1,3], Ken P. Kleinman[1], Matthew W. Gillman[1,4] [1]Department of Ambulatory Care and Prevention, Harvard Medical School/Harvard Pilgrim Health Care, [2]Division of Newborn Medicine, Children's Hospital, Departments of [3]Epidemiology and [4]Nutrition, Harvard School of Public Health; all in Boston, MA

Background. Infants who are small at birth then grow rapidly may be at highest risk for blood pressure elevation later in life. Most studies to date, however, have been limited by lack of accurate length measures. This limitation has necessitated a focus on weight rather than weight-for-length measures, which may be more relevant in terms of adiposity and cardiovascular risk. **Objective.** To examine the effect of growth – in weight-for-length – during the first 6 months of life on systolic blood pressure (SBP) at 6 months of age, and to determine whether the effect of infant growth on SBP varies with size at birth. **Design/Methods.** We studied 642 infants and their mothers from the ongoing prospective cohort study Project Viva. Our main outcome was SBP at 6 months of age, which we measured up to 5 times in each infant with an automated Dinamap monitor. We measured infant length at birth and weight and length at age 6 months with research standard instruments, and we abstracted birth weight from medical records. We derived weight-for-length z-scores (WFL) from the 2000 US CDC growth charts and used mixed effect regression models incorporating each repeated blood pressure measurement. The main exposure variable was WFL at 6 months controlled for WFL at birth, mathematically equivalent to change in WFL from birth to 6 months.



Results. Mean (SD) infant SBP at 6 months of age was 89.7 (13.1) mmHg. Infant SBP was directly correlated with maternal parity and SBP, birth weight and length, and 6-month weight, length, and WFL. Adjusting for WFL at birth and confounding variables, infant SBP was 1.2 mmHg (95% CI 0.3 to 2.1, p=0.01) higher for each 1-unit increment of WFL at 6 months (Figure). The effect of infant growth did not appear to vary across quartiles of WFL at birth (p-value for multiplicative interaction = 0.80)(Figure). *adjusted for WFL at birth, BP measurement conditions, age, sex, gestational age, length at 6 months, and maternal SBP **Conclusions**. More rapid growth in weight-for-length in the first 6 months of life was associated with higher systolic blood pressure at 6 months of age. The effect of infant growth was not appreciably stronger among infants who were smaller at birth.

P3-014

The Effects of a LP Diet During Gestation after STZ Treatment in the Neonatal BalbC Mouse Impairs Pancreatic Regeneration Aaron Cox, Edith Arany, David Hill; Department of Physiology and Pharmacology, University of Western Ontario, Departments of Diabetes and Metabolism, Physiology, Medicine and Pediatrics, Lawson Health Research Institute, University of Western Ontario, London, Ontario, Canada.

Background: Low Protein (LP) diet during pregnancy is a model for intrauterine growth restriction. This causes changes in fetal programming and leads to metabolic diseases later in life, such as Type II Diabetes. It has been shown previously that streptozotocin (STZ) given to a neonatal rat stimulates the pancreas to regenerate. **Objective:** The aim of this study is to determine whether the LP diet will alter the ability of the pancreas to regenerate after STZ injection. **Methods:** Four separate groups of mice were used. Balb/c mice were fed a control (20%) or LP (8%) diet throughout gestation. Mothers were given a control (C) diet during lactation. At birth pups were injected intraperitoneal with (+) or without (-) STZ at 35 mg/kg from days 1 to 5 for each dietary treatment. Female pups were grown up to 22 and 30 days of age, receiving C diet after weaning, and then sacrificed. The pancreas was removed and fixed in 10% formalin and counterstained for insulin and glucagon. The morphometry of the pancreas was analyzed using Northern Eclipse software. **Results:** Preliminary results suggest that treatment with STZ increases regeneration for both C and LP conditions indicated by the increase in the total percentage of islet area at day 22. By day 30, there is still greater islet area for the LP – STZ and the LP + STZ groups compared to the C – STZ and C + STZ groups respectively. LP – STZ has a greater number of large islets at day 30 opposed to the LP + STZ group. The number of small islets is increased at 22 and 30 days of age when compared to the number of large islets for all conditions. There is increased alpha cell area in the C + STZ group at day 22 compared to C – STZ, whereas between the two LP groups there were no changes. At day 30, no change was observed between the C groups, while there was a reduction in alpha cell area for the LP + STZ group in comparison with the LP – STZ group. As well, the Beta cell area for the LP + STZ group in small islets is greater than in the LP – STZ group. **Conclusion:** So far the data suggests that the LP diet does not affect the ability of STZ to stimulate regeneration, as there is an increase in islet cell area for both the control and LP groups. The difference seems to be that regeneration does not occur through an increase in alpha cell area when treated with LP+STZ.

P3-015

Interaction Between Birth Weight and Postnatal Growth Does Not Increase Risk for High Blood Pressure at Age 7 Years : Results from the United States Collaborative Perinatal Project Anusha H. Hemachandra, Penelope P. Howards, Enrique F. Schisterman, Mark A. Klebanoff. Division of Epidemiology, Statistics, and Prevention Research, National Institute of Child Health and Human Development, Bethesda, MD, USA.

Background A physiologic predisposition towards hypertension is theorized to result from rapid catch-up growth in childhood after intrauterine growth restriction. This interaction may be associated with high blood pressure in childhood, which in

turn may predict an increased risk of hypertension as an adult. Rapid catch-up growth is difficult to quantify, but recently the use of changes in weight z-scores has been recognized as a better indication of the increase in age-adjusted size during childhood than gross weight gain alone. **Objective** To use the interaction between birth weight and weight gain during childhood to predict the risk for high blood pressure in childhood, and to identify discreet periods of catch-up growth that put children with intrauterine growth restriction at increased risk for the development of high blood pressure later in life. **Methods** The United States Collaborative Perinatal Project (1959-1974) studied 55,908 pregnancies in an observational cohort at 12 medical centers in the United States, and followed the offspring through age 7 yrs. All white or black children born at full term, who completed the follow-up without kidney or heart disease were included in this post-hoc analysis (n=29,710). High systolic (SBP) and diastolic (DBP) blood pressures at age 7 yrs were defined as BP > 90th percentile. Z-scores were calculated for weight at birth, 4 months, 1 yr, 4 yrs and 7 yrs based on study means and standard deviation, and changes in z-scores for each interval were calculated. Weight at the time of BP measurement was not included in our regression models because of the risk of over-controlling and triggering the "reversal paradox" (Simpson, 1953 and Tu, 2005). **Results** The mean birth weight and gestational age in this population were 3.24 ± 0.48 kg and 39.7 ± 1.4 weeks, respectively. At age 7 years, mean blood pressure for all subjects was 102.1 ± 10.2 mm Hg (SBP) and 61.3 ± 9.8 mm Hg (DBP). Each kg increase in birthweight increased the odds of high SBP by 2.19 (1.92-2.49) and high DBP by 1.82 (1.59-2.08), when race and change in weight z-scores were also included in the regression model. An increase in weight z-score of 1 standard deviation above the previous weight z-score increased the odds of high SBP at 7 yrs by 1.65 (birth-4mo), 1.79 (4mo-1yr), 1.71 (1yr-4yr) and 1.94 (4yr-7yr) in the full model (all significant at the p<.001 level). White race increased the odds of high SBP by 1.51 (1.36-1.66). When subjects were stratified by size at birth (small, appropriate, large), the effect of change in weight z-score did not differ across growth intervals or across models. In an unstratified model, the interaction terms between birth weight and catch-up growth for any of the defined growth intervals were not statistically significant in predicting high SBP. **Conclusions** In this large biracial US cohort, there did not appear to be an interaction between size at birth, postnatal growth, and childhood blood pressure. For each catch-up growth interval, there was a modest but similar increase in the odds of high SBP for each standard deviation of change in relative size. Crossing growth percentiles upward during childhood did increase the risk of high blood pressure, but the magnitude of the effect was not dependent on size at birth.

P3-016

Maternal Nutrition and Paternal Size Affects Age at Adiposity Rebound. The Pune Maternal Nutrition Study (PMNS) Charu Joglekar[1], Chittaranjan S Yajnik[1], Bhalerao A[1], Solat V[1], Chougule S [1], Deokar T[1], Caroline Fall[2] 1. Diabetes Unit, KEM Hospital and Research Centre, Pune, India. 2. MRC Environmental Epidemiology Unit, Southampton, UK

Background: An early age at adiposity rebound (AR) is a risk factor for later obesity and type 2 DM. It is not known what maternal and paternal factors influence AR in the offspring. **Objective**: To investigate maternal and paternal factors influencing adiposity rebound in the offspring. **Study Design**: The PMNS database has information on maternal pre-pregnancy characteristics and her food intake, physical activity and circulating levels of nutrients and metabolites during pregnancy. We also have information on details of breast-feeding and paternal size and socio-economic status. Children were measured serially from birth every 6 months, at last follow up they were 10y old. At 6y body composition was measured by DXA. We defined age at adiposity rebound by age of lowest BMI between 1 and 10 years. **Results:** The average age of adiposity rebound was 6.6 years (sd 1.8), and was similar in boys and girls. Maternal pre pregnant size and weight gain in pregnancy were not related to AR, nor was maternal intake of macronutrients and frequency of micronutrient rich foods (green leafy vegetables, fruits and dairy products). Higher maternal red cell folate and plasma homocysteine concentrations predicted early AR (standardized β=-0.13, p<0.01 and standardized β= -0.14, p<0.01 respectively) but plasma vitamin B12, ferritin, vitamin C concentrations were not related. Lower maternal physical activity during pregnancy predicted early AR (standardized β=0.08, p<0.05). Birth size measurements and period of exclusive and total breast feeding were not predictive but slower growth of skinfolds in infancy predicted earlier AR. Higher socio economic status (standardized β=-0.087, p<0.05) and higher paternal BMI (standardized β=-0.11, p<0.001) predicted early AR. Early AR in these children was associated with higher adiposity (standardized β=-0.08, p<0.05) and higher insulin resistance (standardized β=-0.07, p<0.05) at 6y of age. **Conclusions:** Early AR in rural Indian children is associated with maternal micronutrient status during pregnancy, slower growth in infancy and large paternal body mass. These findings provide important clues to the growing epidemic of obesity and type 2 diabetes in Indians.

P3-017

Aortic Pulse Wave Velocity in the First Year of Life: Inverse Relation with Maternal Blood Pressure in Pregnancy Koudsi A [1], Khan A[1], McElduff P [2], Cruickshank JK [1] [1] Clinical Epidemiology & Cardiovascular Medicine Group, University division of Cardiovascular Sciences, Manchester Royal Infirmary, M13 9WL (clinep@manchester.ac.uk) & [2] Evidence for Population Health Unit, Manchester University, Medical School. Manchester UK

Introduction: The relative contribution in the first year of life of blood pressure (BP), other vascular characteristics and somatic growth to vascular outcomes has not been examined in detail. Early development of arterial vessels may be a key influence on the rise of BP over time. **Aims:** We investigated the influence of standardised measures of maternal blood pressure (BP), anthropometry and glucose tolerance in pregnancy on infant growth, BP and aortic PWV (aPWV). **Methods:** In a prospective longitudinal design, pregnant women were screened at St. Mary's maternity hospital, Manchester, UK. 150 infants were followed for 3 months of which 60 were followed for the first year of life with standardized measures of growth, BP and aPWV. aPWV was measured using an infrared photoplethysmography probe at the aortic arch and a Doppler ultrasound probe at the aortic bifurcation. We studied the interrelationships and trends through time for measurements in infancy and their associations with antenatal maternal factors recorded at 28 weeks gestation **Results:** Maternal weight at 28 weeks of pregnancy was positively associated with infant birthweight and with infant's subscapular, triceps and flank skinfolds during the first year of life (standardized β = 0.22, P = 0.02). Approximately 11% of birthweight could be accounted by paternal weight (P < 0.001) with 10% from reported maternal birthweight (P=0.002). Compared to those in the highest quartile, infants in the lowest quartile of birthweight had the greatest increase in weight between birth and 3 months of age (2390 vs. 2039 g & t = 3.15, P = 0.002) and the lowest increase for the following 9 months of age (4587 vs. 3589 g & t = 3.1, 95% CI 335 to 1660). Maternal SBP at 28 weeks gestation was inversely related to infant weight at birth and 3 months of age (r = -0.18 and r = -0.17, P < 0.04). A strong persisting inverse relation was found between infant aPWV and maternal systolic BP (SBP) (r = -0.6 at birth, r = - 0.6 at 3 months and r= - 0.7 at 12 months, P<0.001 at each time point). Positive correlations of neonatal aPWV were found with birth length, weight and SBP. aPWV at 3 months was inversely correlated with maternal 2-hours glucose tolerance test (r = - 0.17, P = 0.04). Strong tracking was found for infant vascular measurements, notably aPWV ((r = 0.96 for birth and 3 months; r = 0.92 for birth and 12 months, and r = 0.98 for 3 months and 12 months; P<0.001 for all associations). In multiple regression, aPWV at 12 months was significantly related to maternal SBP (β = - 0.5 and P < 0.001), ethnicity (B = 0.4 P = 0.015) and maternal weight (β = - 1.13 and P = 0.01). **Conclusion**: Infant aPWV may be a more precise early index of infant vascular status than infant SBP, as has been found in adults, and is sensitive to the gestational environment marked by maternal SBP.

P3-018

Blood Pressure in Men and Women who Survived the Siege of Leningrad Ilona Koupil, Dmitri B. Shestov, Pär Sparén, Svetlana Plavinskaja, Nina Parfenova, and Denny Vågerö; Centre for Health Equity Studies (CHESS), Stockholm University/Karolinska Institute, Stockholm, Sweden, Institute of Experimental Medicine, Russian Academy of Medical Sciences, St Petersburg, Russian Federation, Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden, University College of South Stockholm (Södertörn), Huddinge, Sweden

Background: Gender differences have been reported from earlier studies of long-term impact of war semi-starvation on mortality. The siege of Leningrad in 1941-1944 was also accompanied by severe starvation. We have extended a previous analysis of men exposed to the siege and investigated its long-term effects on blood pressure, blood lipids, adult height and obesity in both men and women. **Methods**: 3905 men born 1916-1935 and 1729 women born 1910-1940 and resident in St Petersburg (formerly Leningrad) between 1975 and 1982, of whom more than one third experienced the siege, were examined for cardiovascular risk factors in 1975-1977 and 1980-1982 respectively. The effects of siege exposure on body size and cardiovascular risk factors were studied in multivariate analysis stratified by gender and period of birth, with further adjustments for age, smoking, alcohol and social characteristics. Results: Women who were 6-8 year-old and men who were 9-15 year-old at the peak of starvation had higher systolic blood pressure compared to unexposed subjects born during the same period of birth. The respective fully adjusted differences in systolic blood pressure were 8.8, 95% CI 0.1 to 17.5 mm Hg in women and 2.9, 95% CI 0.7 to 5.0 mm Hg in men. A statistical interaction of the siege exposure with gender was significant at p<0.05 in those exposed at ages 6-8 in both age-adjusted and fully adjusted analyses. Mean height of women who were exposed to siege at age 6+ years appeared to be greater than that of unexposed women of same age. The difference was of borderline statistical significance and persisted after adjustments for non-Russian nationality. No consistent effects of exposure to siege on lipid levels or obesity were detected in men or women. **Conclusions**: The experience of severe starvation in childhood and puberty may have long-term effects on systolic blood pressure in surviving men and women with some gender differences in the effect on growth and blood pressure of starvation experienced at pre-pubertal age.

P3-019

Is Infant Growth Related to Burden of Disease in Adulthood? A Systematic Review David Fisher, Janis Baird, MRC Epidemiology Resource Centre, University of Southampton UK, Liz Payne, University of Southampton, Patricia Lucas, Bristol University UK, Jos Kleijnen University of York UK, Helen Roberts, City University London UK, Catherine Law, Institute of Child Health, University College London UK

Objective: To assess the association between infant size or growth and leading causes of burden of disease in adult life. **Methods**: Systematic review using standard methods. **Data source**: Medline, Embase, CINAHL, PsycINFO, bibliographies of included studies, contact with first authors of included studies and other experts. **Main outcome measures**: Leading causes of adult burden of disease selected from the Global Burden of Disease study. **Inclusion criteria**: Studies that assessed the relationship between infant size or growth during the first 2 years and the leading causes of burden of disease in adult life. **Results**: 19 studies (including 12 cohort studies) relating to 10 of the leading causes of burden of disease in adult life met the inclusion criteria. Most studies reported data on infant size, and in relation to mortality. Only 2 studies were set in developing countries and quality assessment indicated that several studies had at least a medium risk of bias in relation to the review question. Larger infant size was associated with reduced rates of IHD in men but not in women (4 studies). Larger size in infancy was associated with increased risk of insulin dependent diabetes (7 studies). Variability in study design and measurements of both infant size/growth and the outcomes restricted the capacity to estimate an overall effect size for relationships. There were considerable gaps in the evidence and many conditions that account for a high burden of disease such as cancer, mental illness, stroke, and chronic obstructive pulmonary disease had few or no studies associating them with infant size or growth. The studies linking infant size with insulin dependent diabetes were from a range of countries and research groups, and contained study subjects born recently (1974-1998). For other leading causes of adult disease, most studies came from a few developed countries, from a limited number of cohort studies, and reported on study subjects born many decades ago (1911-1946). **Conclusions**: There are significant gaps in the evidence relating infant size or growth to leading causes of burden of disease in adulthood. Where there is evidence, our findings suggest that there is no single optimal pattern of infant growth that is associated with beneficial adult health outcomes. The relevance of some of the cohort studies to children growing up now in both developed and developing countries is uncertain. The use of this body of evidence to inform public policy needs to take these uncertainties into account.

P3-020

Effects of Neonatal Rearing Method on Body and Organ Growth, Fat Deposition and Hypothalamic Neuropeptide Y Gene Expression in Goat Kids Masatoshi Matsuzaki, Mitsuru Kamiya and Eisaku Tsuneishi; Department of Animal and Grassland Research, National Agricultural Research Center for Kyushu Okinawa Region, Kumamoto 861-1192, Japan

Background: Little information exists on both immediate and longer term effects of neonatal nutrition on body and organ growth and adiposity. The present study aimed to determine whether neonatal rearing method influences individual organ growth and fat deposition. Furthermore, hypothalamic neuropeptide Y (NPY) mRNA expression was examined as appetite regulatory mechanisms may be programmed by feeding patterns during neonatal period. **Methods**: Twenty-seven newborn male goat kids, balanced according to their maternal parity, litter size and birth weight (3.14 ± 0.09 kg on average), were divided into 3 experimental groups: reared artificially on milk replacer containing either 24 or 28% protein on fresh powder basis (AR24P or AR28P), or reared naturally by their own dams (NR). Artificially reared kids were removed from the dams before suckling and trained to drink at will from an open trough and were offered a cow colostrum preparation for 3 days, and thereafter fed the assigned milk replacer. They had free access to liquid diet in three 15-min meals/day for the first 10 days and in two 15-min meals/day afterwards. The NR kids were housed with their dams and littermate if any and allowed to suckle freely. At 30 days of age, the kids were sacrificed, and organs and internal adipose depots weighed and hypothalamus collected for real-time RT-PCR analysis. **Results**: Neonatal rearing method did not affect overall body growth as evidenced by the similar growth rate (220 ± 6 g/day on average), body weight (9.8 ± 0.2 kg on average) and crown-rump length (61 ± 0.4 cm on average) in 3 groups of kids although umbilical girth was slightly increased in AR compared with NR kids (56 ± 1 vs. 52 ± 1 cm, $P < 0.05$). However, individual organ growth was diversely affected by neonatal rearing method. Both as absolute weight and as relative weight to empty body weight (EBW), AR kids had greater adrenals, kidneys, pancreas, liver and gut compared with NR kids (Table). When expressed as a ratio to EBW, heart was also greater in AR than NR kids (6.8 ± 0.1 vs. 6.0 ± 0.1 g/kg, $P < 0.001$). In contrast, NR kids had strikingly greater amounts of perirenal and gut fat in both absolute and EBW specific basis compared with AR kids (Table). Naturally reared kids had greater brain to liver ratio (0.26 ± 0.02 vs. 0.18 ± 0.004, $P < 0.0001$) and relative carcass weight (580 ± 3 vs. 567 ± 3 g/kg EBW, $P < 0.01$) compared with AR kids. Relative abundance of NPY mRNA did not differ among the experimental groups. Protein content of milk replacer did not affect feed intake of AR kids and no correlation was detected between feed intake and NPY mRNA expression.

Table: Selected organ and adipose tissue weights of goat kids at the age of 30 days

	AR24P	AR28P	NR
Adrenals (g)	0.79 ± 0.04[a]	0.77 ± 0.03[a]	0.61 ± 0.02[b]
Kidneys (g)	79 ± 5[a]	83 ± 4[a]	57 ± 4[b]
Pancreas (g)	8.5 ± 0.5[a]	9.5 ± 0.5[a]	6.6 ± 0.3[b]
Liver (g)	311 ± 11[a]	322 ± 14[a]	240 ± 17[b]
Gut (g)	497 ± 16[a]	496 ± 16[a]	430 ± 27[b]
Perirenal fat (g)	104 ± 13[a]	99 ± 15[a]	167 ± 23[b]
Gut fat (g)	220 ± 23[ab]	195 ± 21[a]	312 ± 41[b]

Means with different superscripts differ at $P < 0.05$

Conclusion: Mother-reared goat kids deposit greater amounts of internal fat at the expense of the growth of several organs as compared with artificially reared kids during first month of life. Changes in organ growth and adipose development caused by different neonatal nutritional management may have significant implications on subsequent growth, adiposity and adult health.

P3-021

Differences in Growth *in utero* in Different Ethnic Groups in Manchester, UK John Oldroyd[1], Abir Koudsi[1], Aysha Khan[2], Janet Dry[2], Fiona Pritchard[2], Avni Vyas[1], Patrick McElduff[4], Peter Clayton[3] and Kennedy Cruickshank[1]; [1]Clinical Epidemiology Group, Manchester University Medical School, [2]St Mary's Hospital, [3]Child Health and Paediatric Endocrinology, Manchester University, Manchester, UK M13 9PT

Background Low birth weight is associated with coronary heart disease and diabetes in adulthood, both of which have higher rates in South Asians than in white European men and women worldwide. Birth weight is a relatively crude measure of growth in utero, but bi-pariental diameter (BPD) and crown rump length (CRL) may be better. We compared growth *in utero* using BPD, CRL and head circumference (HC) between Pakistani and white European origin babies, born locally. **Methods** We recruited normal, pregnant women from antenatal clinics in St Mary's Hospital. Ultrasounds were preformed by ultrasonograhprs or midwives for measures of CRL and BPD at the dating scan (mean (standard deviation) 12.4 (2.3) weeks) and anomaly scan (19.9 (1.6) weeks). HC were measured with carefully standardized methods in neonates within 72 hours of delivery. **Results** In 168 infants (31 Pakistani, 137 European), BPD was marginally higher at the dating scan in Pakistanis than Europeans (2.6 (0.66) *v.* 2.4 (0.51) cm as was HC (10.2 (2.34) *v.* 9.2 (0.9) cm although neither significantly so. This pattern was reversed at the anomaly scan and neonatally. At the dating scan in the whole group, BPD correlated with CRL ($r = 0.902$, $P = 0.098$) as did BPD and HC ($r = 0.985$, $p = 0.001$). Biparietal diameter and HC were correlated at the anomaly scan ($r = 0.28$ $P = 0.001$). No fetal measurement correlated with neonatal weight. Neonatal HC correlated with neonatal weight ($r = 0.703$, $P = 0.001$). **Conclusion** Bi-parietal diameter, CRL and HC may be sensitive markers of ethnic differences in growth *in utero*.

P3-022

Effects of Infant Feeding Method at Two and Four Months of Life on Male and Female Adolescent Leptin Concentrations E.A. Quinn[1], C. W. Kuzawa[1], T. W. McDade[1], and L. S. Adair[2] [1]Department of Anthropology, Northwestern University, Evanston, IL 60208 USA; [2]Carolina Population Center, University of North Carolina, Chapel Hill, NC 27516 USA

Background: In the last several years, the number of known physiological effects of leptin has greatly increased. Leptin is no longer viewed as a satiety hormone, but is a complex, multifunctional hormone with receptors found on many different organs and tissues in the body. A growing number of international studies suggest that leptin levels in Western populations may be unusually high, and the physiological roles of leptin may be played out at very low concentrations. Recent research by Singhal et al. (2004) among a sample of premature infants has suggested that early life feeding method may have effects on leptin sensitivity in later life. However, the effects of infant feeding on leptin sensitivity in full term, free living humans is unknown. In this study, we investigated the effects of feeding style during the first four months of life on leptin concentrations relative to fat mass during adolescence among a sample of Filipino adolescents studied longitudinally since birth. **Methods:** The participants in this study are members of the larger Cebu Longitudinal Health and Nutrition Survey (CLHNS). In 1998, 307 adolescent females and 296 adolescent males were randomly selected from individuals with complete birth histories (n=2056) for a study of cardiovascular disease and metabolic syndrome risk factors. In this investigation, we used regression analyses to model the relationships between plasma leptin concentrations in adolescence and infant feeding style at two and four months of age (classified as exclusive breastfeeding, any breastfeeding - including solid foods and formula - and formula feeding. Plasma leptin concentration was the outcome variable, and triceps skinfold, as a proxy for adiposity, and infant feeding style were predictor variables. Both plasma leptin concentration and triceps skinfold measurements were log transformed and stratified by sex. Triceps skinfolds were divided into sex-specific quartiles, which were used to look at differences in leptin sensitivity at different quartiles of adiposity. **Results:** We identified significant effects of infant feeding

style during the first four months of life on leptin concentrations. Individuals who were exclusively breastfed to two or four months had lower leptin concentrations relative to fat mass at all levels of adiposity. Any breastmilk intake during the first four months of life led to a reduction of leptin relative to fat mass. The effects of breastfeeding on leptin concentrations relative to fat mass increased in strength across sex-specific tricep thickness quartiles. Similar effects of infant feeding style were seen in both males and females, however the effect sizes were stratified by sex. Females had higher fat mass and leptin concentrations compared to males. Higher adiposity was associated with higher leptin concentrations, but these effects were modified by early life feeding style. **Conclusions:** Duration of breastfeeding has an influence on leptin concentration relative to fat mass, especially among individuals with higher levels of adiposity. This suggests that infant feeding style may have long term programming effects on individual sensitivity to leptin, and may contribute to cardiovascular risk factors.

P3-023

Outcomes of Australian Aboriginal Intrauterine Growth Retarded Babies: Aboriginal Birth Cohort 1987-2001 Susan Sayers, Dorothy Mackerras, Gurmeet Singh, Stephen Halpin Menzies School of Health Research, Institute of Advanced Studies, Charles Darwin University Darwin, Northern Territory, Australia

Introduction: Most studies examining nutritional hypotheses relating to the early origins of adult diseases are in western populations where there are low rates of fetal growth retardation. High rates of growth retardation are more likely in developing and indigenous populations where there are practical, geographic and cultural limitations to prospective longitudinal studies. **Aim:** To compare outcomes of Aboriginal babies, small for their gestational age (SGA) to those appropriately grown (AGA), in a population with high rates of SGA[1]. **Methods: Design:** A cross-sectional study within a longitudinal prospective birth cohort study **Setting:** Top End of the Northern Territory of Australia **Subjects:** 462 Aboriginal children who were born at term and examined at a mean age of 11.4 years; 51% were boys and 48% had commenced puberty, 121 were SGA and 341 were AGA. **Outcome measures:** Achieved growth and nutrition, onset of puberty, potential markers of chronic adult disease and hospital admissions since birth. **Results:** The children who were SGA remained significantly shorter and lighter and had significantly smaller head, waist and mid upper arm circumferences than the AGA children. Using NCHS reference standards[2] one-third of children who had been SGA had weight for age Z scores <-2.0 compared to 15% of AGA children. However there were no significant differences in the age of onset of puberty between AGA and SGA children. Neither were there any significant differences between AGA and SGA children in the cross-sectional measures of blood pressure, cholesterol, triglycerides and fasting insulin and glucose, or over the previous 11 years, the number of hospital admissions and the total days in hospital. **Conclusions:** For this Aboriginal population, small babies became small children and compared to appropriately grown children, in the short term, they had no significant disadvantage with respect to past hospitalizations. Furthermore there were no significant differences in the measures of the potential risk factors of chronic adult diseases between these small children and those appropriately grown. These findings suggest that perhaps in the long term, there may be an advantage to remaining small and that low birth weight for gestational age is not the overriding predictor of post neonatal health events. **References** 1. Sayers SM, Powers JP. Risk factors for Aboriginal low birth weight, intra-uterine growth retardation and preterm births in the Darwin Health Region. Aust NZ J Public Health 1997;21:524-530 2. A.G. Dean, T.G. Arner, S. Sangam, G.G. Sunki, R. Friedman, M. Lanting, et al., Epi Info 2000. A database and statistical program for public health professionals for use on Windows 95, 98, NT and 2000 computers. Centers for Disease Control and Prevention Atlanta, Georgia, USA , 2000.

P3-024

Maternal BMI not Maternal Glucose alters Early Childhood Growth Shields BM[1], Knight B[1], Hopper H[1], Hill A[1], Powell R[2], Hattersley AT[1], Wright D[3]; 1. Exeter Family Study of Childhood Health, Peninsula Medical School, Exeter, UK, 2. Research and Development Support Unit, Exeter, UK, School of Mathematics and Statistics, University of Plymouth, Plymouth, UK

Background: Offspring of mothers with gestational diabetes (GDM) are known to be heavier at birth and are more obese in early childhood. It is not known whether this is an effect of maternal hyperglycaemia or maternal obesity, both associated with GDM. We aimed to assess the role of maternal glycaemia and parental BMI in non-diabetic pregnancy. **Methods**: Fasting glucose (mmol/l), weight (kg), height (m) and BMI (kg/m²) were measured in 567 non-diabetic mothers and fathers. Offspring weight (g), length (cm) and BMI (kg/m²) were recorded at birth, 12 weeks, 1 year and 2 years of age. Standard deviation scores (SDS) were calculated for the child measures. Multi-level modelling was used to analyse the longitudinal data. Common determinants of birth weight such as sex, parity, smoking and gestation were added to the model as potential confounders. Results: Maternal glucose was related to child's weight SDS at birth (B= 0.283 [95%CI: 0.077-0.489], p=0.007), but there was no significant association from 12 weeks onwards. Maternal BMI was a strong predictor of child's weight SDS at all four time points (birth: B=0.043 [95%CI: 0.027-0.059], 12wks: B=0.028 [95%CI: 0.012-0.044],1yr: B=0.032 [95%CI: 0.014-0.050], 2yrs: 0.031[95%CI: 0.01-0.052], p<0.005 for all). Paternal BMI had no relationship with weight SDS at birth, but from 12 weeks onwards there was a significant association (12wks: B=0.029 [95%CI: 0.011-0.047], 1yr: B=0.048 [95%CI: 0.027-0.069], 2yrs: B=0.041 [95%CI:0.015-0.067], p<0.002 for all). Further exploration of the data revealed maternal glucose was associated with length SDS at birth and 12 weeks (B=0.28 [95%CI: 0.064-0.496] and B=0.26 [95%CI: 0.061-0.459], respectively, p<0.02 for both) and had a negative association with BMI at 1 and 2 years (B=-0.253 [95%CI: -0.476 - -0.03] and B=-0.288 [95%CI: -0.564 - -0.012], respectively, p<0.05 for both). Maternal BMI was related to length SDS at birth (B=0.018 [95%CI: 0.001-0.035], p=0.04) but not at subsequent time points, and was a strong predictor of child's BMI from birth to 2 years (birth: B=0.048 [95%CI: 0.031-0.065], 12wks: B=0.035 [95%CI: 0.018-0.052], 1yr: B=0.047 [95%CI: 0.028-0.066], 2yrs: B=0.038 [95%CI: 0.016-0.06], p<0.001 for all). Paternal BMI was associated with child's BMI from 12 weeks onwards (12wks: B=0.025 [95%CI: 0.006-0.044], 1yr: B=0.044 [95%CI: 0.022-0.066], 2yrs: B=0.045, [95%CI: 0.019-0.071], p<0.002 for all). **Conclusion**: In non-diabetic pregnancies we have evidence that maternal glycaemia is related to weight at birth and not after 12 weeks. The impact of maternal BMI on offspring weight and BMI persists from birth to 2 years. Increased obesity in offspring of diabetic mothers is therefore more likely to reflect maternal obesity rather than hyperglycaemia. The association of paternal BMI with offspring weight and BMI from 12 weeks onwards may reflect either an early genetic influence, or the effect of early shared environment.

P3-025

Maternal Progesterone Treatment Following Placental Restriction Does Not Alter the Premature Initiation of Lactogenesis, but Improves Postnatal Mammary Gland Development and Pup Growth Andrew L. Siebel, Rachael O'Dowd, Kerryn T. Westcott, Veselin Ceranic and Mary E. Wlodek; Department of Physiology, University of Melbourne, Parkville, Victoria 3010 Australia.

Background: The lactation environment after birth is critical for normal postnatal growth. Recent evidence suggests that accelerated growth in infancy and childhood following fetal growth restriction is implicated as contributing to the increased risk of developing adult diseases. We have shown that placental restriction, which is characteristic of human intrauterine growth restriction, impairs mammary function and pup milk intake in the rat, further compromising postnatal growth. Placental restriction also reduces maternal progesterone concentrations during pregnancy in the rat, which may contribute to the impaired mammary gland development as well as triggering early lactogenesis. Our aim was to determine whether administration of progesterone to placentally restricted rat mothers alters milk protein (beta-casein, alpha-lactalbumin and whey acidic protein (WAP)), progesterone receptor, parathyroid hormone related peptide (PTHrP) and PTH/PTHrP receptor mRNA expression on day 20 of pregnancy in the mammary gland. A second aim was to determine whether the compromised mammary morphology (alveolar area and number) can be rescued during pregnancy (day 20) or lactation (6 days) by exogenous maternal progesterone treatment following placental restriction. **Methods:** Bilateral uterine vessel ligation (Restriction) or sham surgery (Control) was performed on day 18 of gestation in Wistar Kyoto rats (approved by University of Melbourne Animal Ethics Committee). Following placental restriction surgery, pregnant rat mothers were injected with progesterone (1mg/kg, sc) or vehicle and Control mothers with vehicle on the day of surgery, the morning and afternoon of day 19 and morning of day 20. Mothers and pups were killed on either day 20 of gestation or 6 days after birth, with mammary gland alveolar area and number quantified. Maternal mammary gene (beta-casein, alpha-lactalbumin, WAP, progesterone receptor, PTHrP and PTH/PTHrP receptor) expression was quantified on day 20 of pregnancy by real-time PCR. Data were analysed by ANOVA or unpaired t-test (n=8 per group). **Results:** Progesterone treatment restored maternal progesterone concentrations to Control levels. Neither placental restriction nor progesterone treatment had any effect on mammary progesterone receptor mRNA expression on day 20 of pregnancy. However, Restriction surgery alone significantly increased beta-casein, alpha-lactalbumin and WAP mRNA expression by 0.5, 3.4 and 0.75 fold respectively above Control (p<0.05) during pregnancy, with no effect of progesterone treatment. PTHrP mRNA expression followed a similar pattern, whereas there was no significant difference in PTH/PTHrP receptor mRNA expression. Progesterone treatment following placental restriction significantly increased both mammary alveolar number (+60%) and area (+48%) on day 6 of lactation (p<0.05) but not during pregnancy, suggesting enhanced mammary gland development and growth. On day 6 after birth, pups from progesterone treated placentally restricted mothers had significantly higher postnatal body weight, organ weights and dimensions than those from the vehicle treated group (p<0.05). **Conclusions:** In placentally restricted rats, milk protein gene expression was stimulated during pregnancy compared with Controls indicating premature initiation of lactation. Maternal progesterone treatment following placental restriction enhanced mammary development and function during lactation, improving postnatal growth. Hormonal correction of the mammary defect may have beneficial consequences for postnatal growth and hence the development of adult diseases following fetal growth restriction.

P3-026

Liver Programming by How Protein Diet in Early Life Results in Leptin Resistance in Adulthood Sandra Thyssen, David J.Hill and Edith Arany. Department of Medicine, Physiology and pediatrics. Lawson Health Research Institute. University of Western Ontario. London, Ontario. NA6 4V2.

Background: The cluster of pathologies known as metabolic syndrome, including obesity, insulin resistance, type 2 diabetes, and cardiovascular disease, has become one of the most serious threats to human health. However, understanding the molecular mechanisms underlying these individual disorders and their links with each other has been challenging. In previous studies we have shown that a low protein diet (LPD) during gestation and lactation resulted in an altered pancreatic development in the offsprings, a lower birth weight, a reduced ß cell mass at birth and a reduced insulin secretion in later life. At 130 days, visceral fat mass was higher in the male LPD. Intraperitoneal glucose tolerance test (IGTT) indicated that they were insulin resistant. We have shown previously that male LPD rats (130 days) subjected to an insulin challenge and sacrificed after 1 minute showed reduction in muscle and fat phosphorylation of PKB. **Objective**: The aim of this study was to investigate the impact of nutrition in fetal and neonatal rats and its effects on liver development and programming in males and its consequent changes of gene expression in adulthood. **Methods**: Wistar rats were given 20% (C) or 8% (LPD) protein isocaloric diet by compensating with carbohydrates and fat during pregnancy and lactation. At 130 days, male rats were sacrificed, the liver was dissected, snap frozen in liquid nitrogen and total RNA was extracted. Gene microarray was performed using Affimetrix Rat Expression Arrays (RAE230 2.0 GeneChips). **Results**: Liver weight and total protein/DNA content was significantly reduced at all ages in the LPD treated rats ($p<0.05$). The ratio between liver and body weight remained unchanged at all ages and treatments. There were no changes in food intake. By red oil O staining livers of male rats showed abundant small infiltration of fat if compared to the normal controls. The areas of steatosis were basically periportal. In the LPD group, leptin receptor and Glut-2 mRNA in liver were increased when analyzed by microarray and confirmed by real-time PCR. Glut-2 protein was increased when subjected to Western Blot analysis. We also found overexpression of genes related with leptin resistance (glycerol phosphate acyl transferase: GPAT), insulin resistance (Glutaminase, S6 kinase and mamalian target of rapamycin: m-TOR) and insulin signalling (Janus kinase 2, Phospholipase C epsilon 1). **Conclusions**: These data suggest that a low protein diet during gestation and lactation can program the liver and be responsible for the leptin resistance seen in adulthood by increasing plasma levels of leptin receptor and then controlling its biological activity. It has been also postulated that obesity may be a chronic stimulus for endoplasmic reticulum (ER) stress in peripheral tissues and that perhaps ER stress can be a core mechanism involved in triggering insulin resistance in this model.

P3-027

The Influence of Maternal Diet on Postnatal Growth, Development and Physiology [1]Adam J Watkins, [1]Adrian Wilkins, [1]Victoria Pepper, [1]Colm Cunningham, [2]Felino R.A. Cagampang and [1]Tom P Fleming [1]Division of Developmental and Cell Biology. School of Biological Sciences. University of Southampton. Bassett Crescent East. Southampton. UK. SO16 7PX
[2]Developmental Origins of Health and Disease Division (DOHaD), School of Medicine University of Southampton. Princess Anne Hospital. Southampton. SO16 5YA. UK

Alterations in maternal diet during pregnancy have previously been shown to result in altered fetal and postnatal growth, blood pressure, organ allometry and adult behaviour patterns. The aim of this study is to examine the effects of maternal diet during specific windows of gestation on various aspects of postnatal growth and physiology. Virgin female MF-1 mice of defined age/weight range were mated with MF-1 studs. On day of plug, females were assigned to one of three diets for the duration of gestation, (a) 9% casein (low protein diet, LPD), (b) 18 % casein (control diet), (c) 9% casein for the first 3.5 days of gestation (preimplantation period of embryo development) then switched to 18% casein ('switch'). On day of birth, pups were weighed and litters adjusted to a mean size of 6 (3 males, 3 females) and offspring weight were recorded weekly thereafter. Behavioural patterns of offspring were assessed using burrowing, open field and nesting tests as measures of affective behaviour at various ages. At 9, 15 and 21 weeks, tail cuff blood pressure measurements were recorded. At 28 weeks, offspring were sacrificed and organs were removed, weighed and stored for later analysis.
No significant difference in birth weight was observed between control and experimental groups for either males or females. However, both LPD males and females were significantly lighter than 'switch' offspring ($p < 0.05$). Whilst no significant difference was observed between any of the male offspring in their growth patterns for up to 28 weeks of age, 'switch' females became significantly heavier than controls at 4 weeks of age, and remained significantly heavier over the majority of the following 24 weeks ($p < 0.05$). At 15 weeks of age, 'switch' offspring had a significantly elevated systolic blood pressure when compared to controls when analysed either in total or as separate genders ($p < 0.05$). At specific weeks of age, 'switch' and LPD offspring showed significantly elevated open field activities (distance travelled, vertical counts and mean velocity), when compared to control offspring ($p < 0.05$). No significant differences in patterns of nesting or burrowing behaviour were observed between any of the diet treatment groups at any of the times observed. At 28 weeks of age 'switch' and LPD males and females respectively, showed a decreased cerebellum: body weight ratio when compared to control offspring. These data support the hypothesis that alterations in maternal diet during specific windows of gestation result in significantly altered patters of postnatal growth, systolic blood pressure, affective behaviour patterns and organ allomwtry. This research is funded by the HICHD, USA

Maternal Nutrition - Animal

P3-028

Maternal Iron Deficiency Identifies Critical Windows in Cardiovascular Development in the Rat Henriette S. Andersen, Lorraine Gambling, and Harry J. McArdle. Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK.

Background: The "fetal origins" hypothesis states that imbalances in nutritional environment *in utero* can have long term effects such as increased risk of heart disease, hypertension, obesity and Type 2 diabetes[1]. Using our model of Fe-deficiency during pregnancy, we have previously shown that pups are born with lower birth weight, enlarged hearts, and develop increased blood pressure, which persists into adulthood[2]. Identification of the critical periods during development, when the fetus is most susceptible to disturbance in its milieu, is an essential part of understanding the mechanisms. Here, we use our model of Fe deficiency to identify critical windows and to test whether they are different for different outcomes. **Methods:** Female Rowett hooded Lister rats were fed either control (50 mg/kg) or Fe-deficient (7.5mg/kg) diet for four weeks before being mated. Dams were killed on gestation day 10.5 and embryos from the individual dams were randomly selected for culturing in control or Fe-deficient male rat serum. This generated 4 treatment groups: embryos of control dams cultured in control (+/+) or Fe-deficient serum (+/-) and embryos of Fe-deficient dams cultured in Fe-deficient (-/-) or control serum (-/+). They were cultured for 48 h at 37 °C with rotation. After 48 h, the morphology of the embryos and their yolk sacs were evaluated according to the Brown & Fabro scoring system[3]. **Results:** Embryos of control dams cultured under Fe-deficient conditions were significantly smaller ($p<0.05$) with reduced crown rump length (mm) (3.78 ± 0.07 (+/-); 3.94 ± 0.5 (+/+)), yolk sac diameter (mm) (4.1 ± 0.1 (+/-); 4.47 ± 0.07 (+/+)) and fewer numbers of somites (31 ± 0.5 (+/-); 32 ± 0.3 (+/+)) than the controls. Values are mean ± SEM (n=18-24). The inhibition in growth of the (-/-) group was not statistical different from the controls. In contrast, other changes were much more marked. The yolk sac vasculature of the embryos cultured in Fe-deficient serum was less developed than that of the controls (see figure). The vitelline vein was barely visible and the vascular plexus less dense in embryos cultured in Fe-deficient serum. The effect was even more pronounced in the embryos of control dams cultured in Fe-deficient serum. Culturing Fe-deficient embryos in control serum reversed these changes. Values are expressed as mean + SEM (n=18-39). Statistical analysis by Kruskal-Wallis non-parametric one-way ANOVA. The heart was significantly enlarged in Fe-deficient embryos (82.3 + 2.1 pixels (-/-)) cultured in Fe-deficient serum compared to the size of the heart in the control (67.7 + 3.2 pixels (+/+)). Importantly, culturing embryos of Fe-deficient dams in control serum reversed these changes (68.1 + 1.7 pixels (-/+)).
Conclusion: These data are the first to identify periods in embryonic development that are sensitive to maternal Fe deficiency. The major conclusions from these findings are that gestation days 10.5 to 12.5 are critical in the response of the circulatory system to maternal Fe-deficiency. We conclude that there are critical periods during development when supplementation may be more beneficial than others. This may be particular relevant when considering supplementation strategies in humans[4]. **References:** **1**.Barker, DJ. *Eur. J. Clin. Invest.* (1995) 25 (7): 457-63 **2**.L.Gambling et al. *J. Physiol.* (2003) Oct 15;552(Pt 2):603-10.**3**.Brown, NA & Fabro, S. *Teratology* (1981) 24, 65-78 **4**.Cogswell, M E, et al. *Am. J. Clin. Nutr.* (2003)78:773-781. **Funding:** Funded by the International Copper Association.

P3-029

Effects of Maternal Protein Dietary Restriction During Pregnancy on Cyclin G1 Gene Expression in the Murine Fetal and Adult Heart Sanjay Asopa [1,2], Frederick W. Anthony[1], Felino R.A. Cagampang[1], Paul L.Terroni[1], Sunil K.Ohri[2], Mark A. Hanson[1]; [1]Centre of Developmental Origin of Health and Disease, F-887, Princess Anne Hospital, Coxford Road, University of Southampton, Southampton, SO16 5YA. UK; [2]Wessex Cardiothoracic unit, Southampton General Hospital, Southampton, SO16 6YD.UK

Introduction: A nutrient-restricted diet during pregnancy has been shown to be associated with an increase in fetal left ventricular mass (1). Various genes have been identified to be involved in cardiac hypertrophy, but those involved in cell cycle progression from G1 phase to S phase are considered to be important in controlling the mechanism of cardiac hypertrophy. Increased cyclin G1 gene expression in fetal sheep hearts is associated with ventricular hypertrophy (1). The objective of the present study was to measure the cyclin G1 expression in mice hearts during the fetal, postnatal and adult stages after protein restricted diets during pregnancy. **Method:** Pregnant mice were fed either a control diet (18% casein, C) or a protein restricted diet (9% casein, PR) throughout gestation. Offspring hearts at 12 days gestation (C, n=11, PR n=10), postnatal day 1 (C=9, PR=9) and 6 months (C=17, PR=17) life were studied. Whole hearts were extracted and studied from fetal and postnatal day 1 offspring, while left ventricle was dissected out for analysis at the adult stage. Cyclin G1 mRNA expression was measured by reverse transcription and real-time PCR and relative to total RNA, 18s

and ß-actin expression. **Results:** Cyclin G1 expression relative to total RNA was significantly lower in PR murine fetal hearts at day 12 of gestation compared with those from mothers on control diet during pregnancy (C, 23.3±1.7 v PR, 17.0±1.9, p=0.02). Although cyclin G1 gene expression was consistently lower in the postnatal group and adult stage, significant differences were not evident (C, 23.3±1.3 v PR, 22.2± 2.6, p NS; C, 200.7±12.7 v PR, 179.7± 7.9 p=NS, respectively). Similar results were obtained for cyclin G1 expression relative to 18s and ß-actin. A marker of cardiac hypertrophy, caveolin-3, also showed lower mean expression levels at each stage compared with control but these differences did not reach statistical significance. **Conclusions:** A critical effector of cell cycle progression, cyclin G1, was lower in fetal hearts from dams on PR diet during pregnancy. This is the opposite of that found for mature offspring sheep hearts following a 50%global restriction during gestation (1). Whether this reflects species difference or differences in cardiac growth in mid-gestation merits further investigation. **Reference:** Han HC, Austin KJ et al. Maternal nutrient restriction alters gene expression in the ovine fetal heart. J Physiol 2004 Jul 1;558(pt 1):111-21.

P3-030

Endothelium-Independent Mesenteric Vascular Hyperreactivity to Pressors in Hypertensive Prepubertal Microswine Offspring Exposed to Maternal Protein Restriction. Susan P. Bagby, J.B. Roullet, Hong Xue, Kim Saunders. Departments of Medicine, Vascular Surgery, and Comparative Medicine, Oregon Health & Science University and Portland VA Medical Center, Portland OR, USA 97239

Background: Maternal protein restriction (MPR: 1% isocaloric vs 14% protein diet) during the period of nephrogenesis in swine (last 1/3 gestation + 2 wks postnatally) leads to asymmetric growth restriction with accelerated postnatal growth and hypertension without obesity in adult offspring (see related abstract). Prior studies showed increased mesenteric and renal vascular reactivity to AngII despite low AT1R density, suggesting enhanced AT1R signaling pathways. We hypothesized that nutrient deprivation mimics hypoxia and induces compensatory enhancement of fetal oxygen-dependent pathways that persists postnatally, including AT1R-induced NADPH oxidase/H_2O_2 -dependent EGF receptor (EGFR) transactivation. **Methods**. We examined direct arterial blood pressure by telemetry, and vascular contractile reactivity at 3-4 mo of age in prepubertal offspring exposed to 1% MPR (Low Protein Offspring: LPO) vs offspring of 14% maternal protein (NPO). Fresh mesenteric resistance vessels mounted in a wire myograph were exposed i) to 30 uM AngII ± endothelium; ii) with endothelium intact, to AngII ± the specific EGF receptor (EGFR) antagonist AG1478 or the cSrc inhibitor PP2; and iii) to graded doses of KCl, Norepinephrine, and Acetylcholine (Ach). Mean arterial BP (± SD) was increased: 112 ± 9 in LPO vs 102 ± 3 in NPO (n=5, p<.03). Vascular reactivity to AngII was increased in LPO (mean ± SE): 85.8 ± 5.8 mN/mm^2 peak tension (n =7) vs 48.6 ± 6.9 in NPO (n =11; p<.001) and persisted after removal of endothelium. AG1478 blockade of EGFR activation reduced AngII-induced contractile tension in both NPO and LPO but abolished the enhanced AngII response in LPO. Inhibition of cSrc by PP2 induced similar responses. Contractile responses to NE and to KCl were also significantly increased in LPO. Max NE response was increased by 28% in LPO: 139 ± 16 mN/mm (n=7) vs 109 ± 7 in NPO (n = 11); ED_{50} for NE was increased by 66% in LPO: 391 ± 49 nM vs 1146 ± 226 nM in NPO (p<.02). The respective KCl responses in LPO were increased by 44% and 20% (each p <.03). In contrast, vasodilatory responses to Ach and to the NO donor SnP were similar in LPO and NPO groups. Results suggest that the enhanced vascular reactivity to AngII in prepubertal LPO following 1% MPR is independent of endothelium and reflects enhanced signaling through the NaDPH-oxidase-dependent, pro-oxidant pathway reported to transactivate the EGFR. Recent studies in rat aortic vascular smooth muscle cells show that NE also induces NaDPH oxidase activity in vascular tissue (Bleeke et al, Circ Res 2004). Moreover, arterial responses to KCl were increased in a chronic AngII infusion model exhibiting enhanced reactive oxygen species and were blocked by superoxide dismutase (Wang, *Hypertension* 1999). These studies suggest that increased vascular contractile reactivity to NE and KCl observed in LPO may also reflect enhanced signaling through oxidant pathways. If so, our findings in LPO may indicate a generalized postnatal enhancement of oxidant pathways compatible with early nutritional programming of vascular function. This increased vascular reactivity to two major endogenous pressors, already apparent in prepubertal pigs comparable in age to 7-10 y/o children, may contribute to early initiation of hypertension in offspring of maternal protein restriction.

P3-031

Nutritional Restriction in Late but not Early Pregnancy Delays Conception in Guinea Pig Offspring C.E.Bertram[1], O.Khan[1], C.Loades[1], DIW Phillips[2], SG Matthews[3], M.Hanson[1] [1]Centre for DOHaD, University of Southampton, UK., [2] MRC Epidemiology Unit, University of Southampton, UK, Depts of Physiology, Ob-Gyn & Medicine, University of Toronto.

Introduction: Developmental rates, post-natal growth, age at sexual maturity and life span are interrelated in many species. Rapidly developing species mature and reproduce early and have many offspring, but shorter life spans (r-selection) whilst those that develop slowly mature and reproduce later in life, with fewer offspring (K-selection). These strategies may be expected to be influenced differently by prenatal nutrition, but this has not been tested.

In the guinea pig, a precocious species, we have found that pre-natal undernutrition has effects on many body systems such as the hypothalamo-pituitary-adrenal axis, cardiovascular system and sympatho-adrenal system etc. In the present study, we hypothesized that prenatal undernutrition will result in altered reproductive capacity in adult female offspring. **Methods:** Virgin female guinea pigs (F_0) were mated (~450 g) and randomly assigned to one of 3 groups: standard chow *ad lib* throughout gestation (control [C]), 70% of *ad lib* intake from gestational days 1-35 (early [ER]) or days 36-70 (late [LR]). Female (F_1) offspring from each group (n=7 [C], n=7[ER] and n=12 [LR]) were presented to a male at their 2nd oestrus (~ 450g bodyweight), and fed *ad lib* throughout the subsequent pregnancy. Any animal returning to oestrus (cycle 14-16 days) was placed with a male for up to four cycles, after which they were judged to be infertile. **Results**: Whilst there was no difference in age or weight between groups at 1st oestrus, LR guinea pigs did not conceive at their first presentation to the male, and eventually became pregnant when both their age and weight were significantly greater (p<0.01) than either C or ER groups.

Table 1: Age and weight at 1st oestrus and mating in guinea pigs whose mothers were nutritionally challenged during pregnancy. Group data (mean ± SEM) were analysed using one-way analysis of variance (ANOVA; SPSS software). * *p<0.01*

	Control n=7	SEM ±	Early n=7	SEM ±	Late n=11	SEM ±
	Age/days		Age/days		Age/days	
Age 1st oestrus	41.7	1.7	43.3	2.6	42.0	1.8
Age 1st mating	56.7	1.9	54.0	4.5	54.7	1.5
Age successful mating	85.0	13.7	92.7	23.6	**164.8***	11.8
	Wt/g		Wt/g		Wt/g	
Weight 1st oestrus	389	27.5	367	18.1	372	14.5
Weight 1st mating	479	28.4	494	21.7	452	31.4
Weight successful mating	658	55.6	686	36.3	**814***	20.2

Conclusion: Our finding of a delay in successful conception in the LR offspring group does not appear to be due to delayed onset of oestrus, nor the need to attain a critical weight to become pregnant. Either an alteration to the hypothalamic-pituitary-gonadal axis or behavioural changes, making the LR group females less receptive to the male are likely to be involved. This research was supported by a grant from the British Heart Foundation (MAH & SGM)

P3-032

Periconceptional Undernutrition and Twin Size both affect Growth and Metabolic Responses of Twin Fetal Sheep to an Acute Maternal Fast in Late Gestation Frank H Bloomfield, Chris Rumball, Mark H. Oliver, Anne L. Jaquiery and Jane E. Harding, Liggins Institute, University of Auckland, Auckland, New Zealand.

Background: We have previously shown that in late gestation singleton fetuses, fetal growth trajectory, and growth and metabolic responses to a late gestation maternal fast, depend upon nutritional status around the time of conception. The fetal growth trajectory of twins may also be determined early in gestation. We therefore hypothesised that in twin fetuses, growth and metabolic responses to a late gestation maternal fast would be affected by both maternal periconceptional nutrition and fetal twin growth status , (heavier (H) vs lighter (L) twins of a pair). **Methods:** Romney ewes were randomly assigned to *ad libitum* feed (N, *n*=5) or undernutrition (UN, ewe weight reduced by 10-15%, *n*=9) from 60 d before until 30 d after mating (term = 147 d). Vascular and chest girth growth catheters were implanted in both fetal twins of each pair at 110 d gestation. Growth was measured twice daily. On d 121 of gestation feed was removed and the ewes were fasted for 3 d. On d 124 of gestation the ewes were refed and simultaneously received an intravenous infusion of 25 g glucose over 8 hours. Blood samples were withdrawn before and twice daily during the fast and at 2, 4, 6, 8, 24 and 48 hr after refeeding. Data were analysed by repeated measures ANOVA with nutritional group and twin growth status (heavier (H) vs lighter (L) as covariates, and are presented as mean±SEM. **Results:** (See table for metabolic data). Pre-fasting, there were no significant differences in growth rate, PaO_2, glucose or urea concentrations between nutrition groups, but lactate concentrations were lower in UN fetuses. During the fast, glucose and lactate levels were lower and urea levels higher, in UN fetuses. There were significant interactions between nutrition group and twin growth status for lactate throughout the study, and for glucose during refeeding. During the fast and refeed, PaO_2 levels were lower in UN fetuses (22.4 vs 25.2±0.7 mmHg, *P*=0.002) with a significant twin growth status interaction (N(L) having the highest levels). After the onset of the maternal fast, growth trajectory was less in UN twins (5.3±0.2 vs 5.6±0.1 mm/d, *P*=0.01) and was significantly greater in H vs L twins (*P*=0.003).

	Pre-fast metabolites (mM)			During fast (mM)			During refeeding (mM)		
	glucose	lactate[¶]	urea	glucose*	lactate[¶]	urea*	glucose[†]	lactate[†]	urea
N(H)	0.9±0.0	1.3±0.1	5.4±0.3	0.6±0.0	1.4±0.0	7.7±0.3	0.8±0.0	1.5±0.0	7.3±0.4
N(L)	0.8±0.0	1.6±0.2	5.3±0.3	0.6±0.0	2.1±0.3	7.8±0.3	0.9±0.1	1.8±0.1	7.4±0.4
UN(H)	0.8±0.0	1.4±0.1	5.5±0.2	0.5±0.0	1.5±0.1	8.3±0.3	0.9±0.0	1.9±0.1	7.7±0.3
UN(L)	0.8±0.0	1.1±0.1	5.5±0.2	0.5±0.0	1.3±0.0	8.3±0.3	0.8±0.0	1.5±0.0	7.7±0.3

$P<0.05$ for nutrition group, †$P<0.01$ for nutrition group 5 twin growth status interaction.

Conclusions: Both periconceptional undernutrition and twin growth status affect the fetal metabolic responses to a maternal fast / refeed in twins. Growth slows in

UN twins and in N(L) twins, with the increase in PaO_2 in the latter suggesting decreased oxidative metabolism. In UN twins, elevated urea levels suggest increased mobilisation of protein, perhaps in response to the lower glucose levels. However, the metabolic response during refeeding is determined by an interaction between periconceptional nutritional status and twin growth status, suggesting possible reasons why metabolic studies in twins may produce conflicting results. We suggest that more detailed careful studies in twins, utilising techniques such as within-pair analysis, are necessary to unravel the complex interactions between twin growth status and maternal effects

P3-033

Effect of Periconceptional Undernutrition on Insulin Responses to Glucose and Arginine Stimulation in Late Gestation Twin Fetal Sheep Frank H Bloomfield, Chris Rumball, Mark H. Oliver, Anne L. Jaquiery and Jane E. Harding, Liggins Institute, University of Auckland, Auckland, New Zealand.

Background: Small size at birth is associated with an increased risk of glucose intolerance later in life. We have previously shown that periconceptional undernutrition in singleton sheep reduces fetal growth trajectory and alters insulin responses to glucose and arginine stimulation in late gestation. Twins are universally born small, but the human studies are conflicting regarding their risk of glucose intolerance in later life. We hypothesised that both periconceptional undernutrition and within pair fetal size in twins would affect insulin responses to glucose and arginine stimulation tests. **Methods:** Romney ewes were randomly assigned to *ad libitum* feed (N, *n*=5) or undernutrition (UN, titrated to reduce ewe weight by 10-15%, n=9) from 60 d before until 30 d after mating (term = 147 d). Vascular and growth catheters were implanted in both twins of a pair at 110 d gestation. Both twins underwent an intravenous glucose tolerance test simultaneously (GTT; 0.5 g/Kg estimated fetal weight) and an intravenous arginine challenge simultaneously (arg; 100 mg/Kg estimated fetal weight) on day 118 of gestation. Ewes were fasted the previous night and the tests were separated by at least 4 hours. Blood samples were withdrawn simultaneously from both fetuses at 0, 2, 5, 10, 15, 30, 45 and 60 minutes after injection. Glucose concentrations were measured on an autoanalyser, arginine by HPLC and insulin by radioimmunoassay. Glucose and arginine levels were compared by repeated measures ANOVA, and areas under the curve (AUC) by ANOVA, with nutritional group and heavier / lighter twin status as covariates. Insulin values were compared by factorial ANOVA due to missing datapoints, with time as an additional covariate. Data are mean±SEM. **Results:** GTT: glucose levels and glucose AUC following glucose injection were not different between nutritional groups (glucose AUC: N 92±3, UN 88±2 mM.min). Peak insulin responses (at 15 min) were not significantly different (N 0.9±0.1, UN 0.7±0.1 ng/mL), but overall insulin response was significantly lower in UN fetuses (P=0.008) and tended to be lower in lighter twins (P=0.08). Arginine test: arg levels and arg AUC were not different between nutritional groups (arg AUC: N 11.9±0.4, UN 11.2±0.3 mM.min). Peak insulin concentration in response to arginine was not significantly different between groups (N 0.4±0.1, UN 0.3±0.1 ng/mL), but overall insulin response was significantly lower in UN fetuses (P<0.0001) and in lighter twins (P=0.04). However, glucose levels were also significantly lower in UN fetuses (P=0.007), with glucose AUC also lower (N 36±2, UN 32±2 mM.min, P<0.1). There were no interactions between nutritional group and twin growth status for any of the parameters in either test. **Conclusions:** Periconceptional undernutrition reduces fetal insulin response to both a glucose load and to arg stimulation in twins. Lighter twins also have a lower insulin response, independent of nutritional group. These data suggest that UN twins have both reduced pancreatic response to glucose and reduced beta cell insulin storage. The lower glucose concentrations after arginine challenge and similar glucose disposal curves following glucose injection in the face of lower insulin concentrations in UN twin fetuses suggest that periconceptional undernutrition increases peripheral sensitivity to insulin.

P3-034

The Effect of Pre- and Peri-conceptional Undernutrition on Coronary Artery Velocity in Adult Male Sheep Offspring Julian P. Boullin[1,2], Arthur M. Yue[2], Jane K. Cleal[1], Lucy Braddick[1], Deborah Burrage[1], David E. Noakes[3], John M. Morgan[2], Mark A. Hanson[1] & Lucy R. Green[1].1. Centre for Developmental Origins of Health and Disease, University of Southampton, Southampton, United Kingdom. 2. Wessex Cardiothoracic Unit, Southampton University Hospitals NHS Trust, Southampton, United Kingdom. [3]Department of Veterinary Reproduction, Royal Veterinary College, AL9 7TA

Introduction: It is well recognised that size at birth is linked to cardiovascular disease (1). In sheep that were made anaemic *in utero* coronary flow is increased in adulthood (2). We used a sheep model to investigate whether nutritional challenges around time of conception influences the coronary artery velocity (CAR) in adult male sheep. **Methods:** Welsh Mountain ewes (UK Animals (Scientific Procedures) Act 1986) were assigned to dietary groups prior to mating and fed 100% total nutrient requirements (Control, n=6) or 50% total nutrient requirements 30 days prior to conception (PRE, n=8) or for 15 days before and after conception (PERI, n=10) and 100% thereafter. Male offspring were weaned at 13 weeks and then fed standard diet *ad libatum*. In male offspring at 3.3 years under general anaesthesia (2% halothane in oxygen), resting and maximal coronary velocity (adenosine induced hyperaemia) and coronary artery velocity reserve (CAVR) were measured with an intravascular doppler guide wire in the left anterior descending coronary artery (absolute CAVR=maximal CAV-resting CAV; relative CAVR=maximal CAV/resting CAV). Data (mean ±S.E.M.) were analysed by ANOVA and a Bonferroni post-hoc test.

Results

	Resting CAV	Maximal CAV	Absolute CAVR	Relative CAVR
CONTROL	8.87±0.83	31.56±1.01	22.69±1.45	3.71±0.35
PRE	9.93±0.88	34.72±1.43	24.79±1.05	3.69±0.27
PERI	9.34±0.62	37.33±1.48*	27.98±1.43*	4.10±0.27

**P<0.05, significantly different from control group*

Conclusion: This data shows that periconceptional, but not preconceptional, undernutrition increases maximal CAV and absolute CAVR. This is the first study to show that coronary circulation is influenced by undernutrition in the periconceptional period and that the effects persist into adulthood. The adaptive consequences of this effect are not known. *Supported by The British Heart Foundation* **Reference List** 1. Barker D 1998 Mothers, Babies and Health in Later Life. Churchill Livingstone, pp 1-217 2. Davis L *et al.* 2003 Augmentation of coronary conductance in adult sheep made anaemic during fetal life. J Physiol 547:53-59

P3-035

Maternal Nutrient Restriction in Late Gestation Upregulates mRNA Abundance for the Insulin Receptor and Peroxisome Proliferator-activated Receptor Gamma but not Interleukin 6 in Adipose Tissue of Newborn Sheep E.A.Butt, A. Latunde-Dada, M. Hyatt, S Pearce, T. Stephenson, M.E. Symonds and H Budge Centre for Reproduction and Early Life, Institute of Clinical Research, University of Nottingham, NG7 2UH UK

Introduction Maternal nutrient restriction targeted over the final month of gestation results in a reduced fat mass in the newborn but greater fat deposition in the resulting offspring (Gardner et al. 2005). These adaptations may be mediated in part by increased sensitivity of fat in the newborn to insulin although this has yet to be established. Insulin also regulates fat growth by stimulating peroxisome proliferator-activated receptor gamma (PPARg), whilst increased interleukin-6 (IL6) is associated with obesity and accompanying macrophage accumulation within fat. The aim of this study was to determine whether maternal nutrient restriction in late gestation results in enhanced insulin sensitivity or IL6 abundance in adipose tissue in the newborn. **Methods** Eighteen pregnant sheep of similar age, body weight and condition score were entered into the study of which nine were nutrient restricted (NR - 50% of metabolisable energy) from 110 days gestation until term (145 days) and nine (controls; C) were fed to requirements. All lambs were born spontaneously and were humanely euthanased at one day of age to enable perirenal adipose tissue sampling. The relative abundance of insulin receptor (IRß), PPARg mRNA to 18S rRNA were determined using RT-PCR whilst IL6 was determined by real-time PCR. All results are expressed as means with standard errors. **Results** The mRNA abundance for all three genes examined were significantly upregulated in the NR offspring compared to C i.e. IRß - NR 179 ± 18; C 33 ± 4 arbitrary units (a.u.) (p<0.01); PPARg - NR 112 ± 8; C 67 ± 4 a.u. (p<0.05) whereas IL6 NR 0.56 ± 0.30; C 0.71 ± 0.26 fg/mcl (NS); was unaffected. In conclusion, maternal nutrient restriction coincident with the period of maximal fat deposition in the fetus increases insulin sensitivity within the adipocyte in conjunction with an increase in abundance of adipogenic genes but not inflammation-related IL-6 expression. **References** Gardner DS, Tingey K, van Bon BWM, Ozanne SE, Wilson V, Dandrea J, Keisler DH, Stephenson T, and Symonds ME. Programming of glucose-insulin metabolism in adult sheep after maternal undernutrition. *Am J Physiol*: doi:10.1152/ajpregu.00120.02005, 2005.

P3-036

A High Unsaturated Fat, High Protein and Low Carbohydrate Diet During Pregnancy and Lactation Modulates Hypothalamic Leptin Receptor Gene Expression in the Murine Adult Affspring Felino R.A. Cagampang, Paul L. Terroni, Frederick W. Anthony, Junlong Zhang, Mark A. Hanson and Christopher D. Byrne; Centre for Developmental Origins of Health and Disease, University of Southampton, Princess Anne Hospital (F-887), Coxford Road, Southampton SO16 5YA, UK

Introduction. The hormone leptin, which is mainly produced in adipose tissues, is involved in energy homeostasis by inhibiting food intake and in the stimulation and maintenance of energy production. It does this by regulating the expression of neuropeptides involved in appetite regulation via activation of the long form of the leptin receptor (Ob-Rb) in neurons within the hypothalamic region of the brain. Changes in the hypothalamic level of expression and/or a shift in leptin receptor dynamics could therefore modulate food intake and contribute to the development of obesity. Many obese individuals trying to lose weight have turned to diets low in carbohydrates, high in lipid and protein. Inadvertently, some individuals may be eating this diet during pregnancy. There is therefore a need to determine the long-term effects on the offspring of maternal consumption of such diets. The aim of the study was to determine whether hypothalamic Ob-Rb gene expression in the adult offspring was affected by a maternal diet high in fat and protein during pregnancy and lactation. **Methods.** Virgin female Balb/C mice (3 weeks old) were randomly assigned to two dietary groups. They were fed *ad libitum* with either a low carbohydrate (CHO), high fat and high protein (HFP; n=9) diet containing 36.8% CHO, 32% lipid and 28% protein respectively, or a standard laboratory chow diet

(n=6) containing 68.8% CHO, 10% lipid and 18% protein, for 6 weeks prior to conception. At 9 weeks old, all animals were time-mated and pregnancy was determined by the presence of vaginal plug (defined as day 0). The diet assigned to an animal prior to conception was also given throughout the gestation and lactation period. All offspring were weaned at 3 weeks of age onto the standard chow diet and maintained on this diet *ad libitum* until they reached adulthood. At 8 weeks old the offspring were killed and brain blocks containing the hypothalamus were collected and analyze for changes in the level of gene transcripts for Ob-Rb by reverse transcription and real-time PCR. The results were expressed relative to 18s ribosomal RNA. **Results.** There was a 45% reduction (chow vs HFP: means±SEM of 1.03±0.20 vs 0.57±0.04 respectively, $p<0.01$) in Ob-Rb mRNA levels in hypothalamic tissues of adult male offspring from mothers fed the HFP diet during pregnancy and lactation compared to those exposed to the chow diets. However in the female offspring no difference in hypothalamic Ob-Rb mRNA levels was observed (chow vs HFP: 1.07±0.05 vs 1.04±0.13, p=0.84). **Conclusions.** We have found sex-specific differences in the effect of a maternal diet enriched with unsaturated fat and protein during gestation and lactation on hypothalamic Ob-Rb mRNA expression in the male adult offspring. These changes may be involved in modulating appetite and contribute to the development of obesity in later life. The factors which prevent such reduction in Ob-Rb gene expression in the adult female offspring remain to be determined. *Supported by the British Heart Foundation*

P3-037

Effect of High Fat Maternal Diet in the Fetal Programming of Metabolic Disorders Kanta Chechi, John J. McGuire*, Sukhinder Kaur Cheema; Department of Biochemistry, Division of Basic Medical Sciences*, Memorial University of Newfoundland, St. Johns, Newfoundland, A1B3X9-Canada.

Background: A high maternal dietary fat intake during pregnancy has been shown to be associated with the "fetal programming" of adult metabolic disorders in the offspring. However, the possible mechanisms that enhance the susceptibility of developing fetus to future disorders are still unknown. We propose that high fat maternal diet interferes with the programming of genes related to lipid metabolism, which leads to the development of future disorders in the offspring. Another important issue is the role of different dietary fats in the fetal programming, as saturated fatty acids (SFA) are considered deleterious whereas polyunsaturated fatty acids (PUFA) are known to be beneficial. We further propose a possible role for the interaction of offspring's own diet with the programming due to maternal diet. **Methods:** Female C57BL/6 mice were fed a high fat diet (20% fat w/w) rich in either saturated (SFA) or polyunsaturated fats (PUFA), for two weeks before mating and during pregnancy until weaning. Their offspring were divided into two groups; each group was fed high fat diets enriched in either SFA or PUFA until 8 weeks of age. Blood, bile and tissues were collected at the end of 8 weeks. This experimental design, where pups from mothers fed diets high in SFA were exposed to either SFA or PUFA and vice versa, enabled us to delineate the individual effects of different dietary fats. We could further gain an insight on the interaction of maternal diet with that of the pup's own diet. To assess vascular dysfunction associated with metabolic syndrome, KCl, phenylephrine-, and thromboxane A_2 mimetic U46619-induced contraction and both the acetylcholine (endothelium-dependent)- and sodium nitroprusside (endothelium-independent)-induced relaxation of isolated aortic rings from these female pups were measured. **Results:** Our initial observations show reproductive failure in the female mice on high fat diet resulting in only 40% pregnancy rate. The plasma profile of female pups suggest that level of triglycerides (TG), HDL-cholesterol, LDL-cholesterol and total-cholesterol were significantly higher in the pups born to mothers on SFA diets, who continued on SFA diets (SFA/SFA) as compared to all other groups. The TG, HDL-, LDL- and total-cholesterol concentrations were significantly lower in pups from mothers on PUFA who continued on PUFA (PUFA/PUFA). The pups on cross-over diets i.e SFA/PUFA and PUFA/SFA showed low TG levels but HDL-, LDL- and total-cholesterol values were in between SFA/SFA and PUFA/PUFA. KCl-, phenylephrine- and U44619-induced contractions of aorta were reduced in all the groups as compared to the control mice; female pups born to chow-fed mothers that continued feeding on chow. Surprisingly, maternal PUFA treatment alone was sufficient to reduce both endothelium-dependent and –independent relaxation responses of aorta from female offspring. **Conclusions:** Our data suggest an effect of maternal dietary fat intake on the plasma lipid profile and aortic smooth muscle function of the offspring, which could be due to the altered metabolic pathways in the offspring. Nevertheless these data further suggest that a pup's own diet also influenced the programming effects of the maternal diet.

P3-038

High Intakes of Multi-Vitamins during Pregnancy in Rats Alters Growth and Food Intake Regulation in the Offspring Paul J. Das, Ignatius M.Y. Szeto, Rania Abou Samra, Nobuhiko Okubo and G. Harvey Anderson. Department of Nutritional Sciences, University of Toronto, 150 College St, Toronto, Ontario, M5S 3E2

Background: Multi-vitamins are the most common supplement consumed by women, especially during pregnancy. Because vitamins have been shown to play a key role in epigenetic modifications of development in utero, we hypothesized that high multi-vitamin intake during pregnancy in rats is reflected in the growth and food intake regulation of the offspring. **Methods:** In two experiments, two groups of pregnant Wistar rats were fed the AIN-93 diet containing either the required (RV) or 10x higher multi-vitamin (HV) content. Male offspring (n=10/group) were weaned to either a standard AIN-93 diet (S) or an obesity-inducing liquid diet (Ob). In experiment 1 (Expt 1), body weight (g) was measured weekly from weaning to 15 wks of age. At 15 wks and after an overnight fast, offspring received either a glucose (5 g/kg) or water preload given by gavage in random order on alternate days. Food intake (FI) suppression was determined by the difference in 1 hr FI measured 30 minutes after the preloads. On a separate day, plasma GLP-1 and ghrelin were measured in fasted pups 30 minutes after a glucose load. In Expt 2, using the same design, body weight (g) was measured weekly from birth to weaning. At weaning, FI suppression in response to glucose (5 g/kg) and whey protein (5 g/kg) preloads was assessed. **Results:** Maternal HV diet did not affect birth weight (8.3 ± 0.2 vs. 8.3 ± 0.3 g). However, at weaning, offspring born to HV mothers weigh less than those born to RV mothers in both Expt 1 and 2 (65.4 ± 0.8 vs. 70.5 ± 1.2 g; P<0.01 and 68.7 ± 0.6 vs. 72.1 ± 1.0; P<0.01, respectively). At weaning, the HV maternal diet affected FI response to glucose but not to whey preloads. After glucose, offspring born to HV mothers reduced FI more than those born to RV mothers, but this effect was only in those fed the Ob diet (-1.6 ± 0.4g vs. 0.2 ± 0.3 g, P<0.01). At 15 wks, there was a main effect of pup diet and an interaction between maternal diet and pup diet on body weight gain from weaning. Pups fed the Ob diet gained more weight than those fed the S diet (644.7 ± 15.3 vs. 555.3 ± 14.7 g; P<0.01). Also, pups fed the S diet and born to HV mothers gained more weight over the 15 wks than those born to RV mothers (497.7 ± 14.7 vs. 458.5 ± 7.4 g) but there was no effect of maternal diet on pups fed the Ob diet. There was a significant interaction between maternal diet and pup diet on FI suppression and hormone response to glucose at 15 wks. Offspring fed the S diet and born to HV mothers compared to those born to RV mothers decreased FI less (-0.8 ± 0.2 vs. 1.7 ± 0.4 g, P<0.01), and at 30 min after the glucose load had lower GLP-1 (3.6 ± 0.2 vs. 5.1± 0.5 pM, P<0.01) and higher ghrelin (1705 ± 93 vs. 1412 ± 73 pg/ml, P<0.05) concentrations. Conversely, offspring fed the Ob diet and born to HV mothers compared to those born to RV mothers decreased FI more (-9.9 ± 1.2 vs. -3.8 ± 0.5 g, P<0.01), and at 30 min after the glucose load had higher GLP-1 (7.1 ± 0.8 vs. 4.9 ± 0.6 pM, P<0.01) and lower ghrelin (1172 ± 36 vs. 1545 ± 129 pg/ml, P<0.05) concentrations. In summary, the HV maternal diet led to a smaller reduction in FI after glucose and increased weight gain in the offspring fed a standard diet but a greater reduction in FI when the offspring were fed an obesity-inducing diet and was protective against excess weight gain. **Conclusions:** High intakes of multi-vitamins during pregnancy affect food intake regulation in the male offspring, but the effects are also dependent on the pup diet. Research funded by NSERC & BMS/MJ Freedom to Discover Grant.

P3-039

Differential Signaling of Growth Pathways in Fetal Liver and Muscle in Response to Maternal Nutrient Restriction Min Du[1,2], Qingwu W. Shen[1,2], Hyungchul Han[1,2], Mei J. Zhu[1,2], Michelle M. Schwope[1,2], Warrie J. Means[1,2], Peter W. Nathanielsz[1,3], and Stephen P. Ford[1,2] [1]Center for the Study of Fetal Programming, [2]Department of Animal Science, University of Wyoming, Laramie, WY 82071; [3]Center for Pregnancy and Newborn Research, University of Texas, Health Sciences Center, San Antonio, Texas 78229 USA

Background: Our model of early to mid gestational nutrient restriction in the ewe induces a marked IUGR (~30%). While fetal liver weight was a greater % of fetal weight in nutrient restricted (NR; n=7) than in control fed (CF; n=6) ewes (6.7 ± 0.4 vs 6.0 ± 0.1%, mean ± SEM; P<0.05), *longissimus dorsi* (Ld) muscle weight as a % of fetal weight remained unchanged (1.1 ± 0.1 vs 1.0 ± 0.1%). The mechanisms responsible for the differential effects of undernutrition on fetal liver and skeletal muscle growth and development are unclear. The mammalian target of rapamycin, mTOR, is a key mediator of protein synthesis and is stimulated by nutrient availability. Growth factors, like IGF-1, activate IGF receptor which activates the phosphoinositide 3-kinase (PI3-K) /protein kinase B (Akt) pathway, leading to mTOR activation through phosphorylation. Phosphatase with tensin homology (pTEN) is a phosphatase that negatively regulates the PI3-K /Akt pathway. We hypothesized that nutrient restriction would induce the down-regulation of pTEN in fetal liver but not fetal skeletal muscle which could explain the differential impacts of undernutrition on liver and Ld muscle growth. The objective of this study was to elucidate the effect of maternal nutrient restriction on pTEN and associated Akt/mTOR signaling in fetal liver and Ld muscle. **Methods:** Ewes were fed to 50% (NR group) or 100% (CF group) of total digestible nutrients (NRC requirement) from 28 to 78 days gestation. On Day 79 of gestation, fetal liver and Ld muscle tissues were collected and snap frozen for later analyses. **Results:** Nutrient restriction reduced the amount of phosphorylated IGF Type 1 receptor (IGFR) in fetal liver (CF 0.61 ± 0.02 and NR 0.50 ± 0.05; P ≤ 0.05), indicating decreased fetal hepatic IGFR signaling in the presence of nutrient restriction. No difference was detected in phosphorylation of Akt (Ser^{473}), mTOR (Ser^{2448}) and ribosomal protein S6 ($Ser^{235/2369}$) in fetal liver, suggesting that fetal hepatic Akt signaling was unaffected. Content of pTEN, was reduced in fetal liver in the NR group (CF 1.08±0.11 and NR 0.68±0.02; P≤0.05); a similar reduction in the activity of pTEN was also observed in NR fetuses. In contrast, in fetal skeletal muscle mTor signaling was down regulated (43%) during NR, and neither pTEN content nor activity was altered by nutrient restriction. This reduction in pTEN content and activity in NR fetuses may have promoted the PIP3/Akt signaling in fetal liver and might be the main reason for the lack of difference in Akt signaling despite a reduction in IGFR activation. These results suggest that there is a differential regulation of growth signaling between fetal liver and skeletal muscle

under maternal nutrition restriction. **Conclusions:** To our knowledge, this is the first report that fetal liver pTEN content and activity are altered by nutrient availability. Since PI3-K/Akt/mTOR signaling is one of the main pathways driving protein synthesis in response to growth factors and nutrients, reduction in pTEN content and activity in fetal liver but not skeletal muscle may partially explain the increase in relative fetal hepatic but not skeletal muscle weight during NR.

P3-040

Catch-up Growth, Body Composition, and Stress-dependent Hypertension in Juvenile Offspring of Maternal Protein Restriction in Microswine. Elizabeth DuPriest[1], Baoyu Lin[2], Philipp Kupfer[2], Kaiu Sekiguchi[2], Jonathan McNeil[2], Lori Woods[2], Kim Saunders[3], Susan Bagby[1,2]. Depts of [1]Physiology & Pharmacology, [2]Med/Div of Nephrology & Hypertension, [3]Comparative Medicine, Oregon Health & Science Univ., Portland VAMC, Portland, OR, USA.

Microswine offspring of maternal protein restriction exhibit asymmetric growth restriction, followed by catch-up growth and hypertension by 6-mo (adult). We hypothesize that in growth-restricted offspring, development of excess body weight relative to their permanently reduced nephron number (via catch-up growth) results in single-nephron hypertrophy and hyperfiltration with accompanying hypertension. **Methods**. To define timing of catch-up growth and hypertension onset in our model, we serially studied Normal- (NPO) and Low Protein Offspring (LPO) over 0-4 mo, including body composition by dual energy X-ray absorptiometry (DEXA), arterial blood pressure (BP) by telemetry, and body and organ weights. **Results.** Body weight (BWt) was decreased non-significantly at birth; at the end of the protein restriction period (2 wks of age), LPO (n=10) weighed significantly less than NPO (n=12): 2.82±0.20 (mean±SD) vs 2.28±0.44 kg; p=.001). Based on z-score plots, LPO males exhibited greater reduction in BWt at 2 wks than LPO females compared to respective sex-matched controls. All LPO exhibited accelerated growth between 2-11 wks (p<.0001); LPO males caught up to sex-matched controls in BWt at 82 days, compared to 40 days for LPO females. Sys BP (but not Dias or MAP) at 3 mo was significantly increased in LPO (n=7) compared to NPO (n=10), but only on post-surgery days 1-2: Sys 140±13 (mean±SD) vs 131±7 mmHg, p<.04; Dias 91±12 vs 88±10, pNS; Mean BP 112±11 vs 107±9 (pNS). All BP indices in LPO returned to control levels by PO days 4-6. Body composition was assessed during accelerated growth (5 wks) and after completion of catch-up (3 mo). At 5 wks, LPO (n=9) had reduced central (truncal) adiposity: 0.115±.14 vs 0.155±.03 g trunk fat mass (FM)/g total fat-free mass (FFM) in NPO (n=12) (p=.01). Females (n=7) had higher F:FFM ratio than males (n=14). Also at 5 wks, FFM(gm):current length (cm)(latter unaffected by maternal diet) trended low in LPO males vs normal males but high in LPO females (sex-diet interaction, p=.01). At 3 mo, body F:FFM ratio in LPO males did not differ from NPO males; the single female LPO at 3 mo was obese cf NPO females, but low n precludes conclusions. Also at 3 mo, total body F:FFM ratio and central adiposity were increased in females (n=6) vs males (n=8) (p=.0003 and p=.0005, respectively). FFM:current length did not differ between diet or sex groups at 3 mo. We propose that, during protein restriction, male LPO, as compared to female LPO, exhibit greater reductions in fat-free mass with similar reductions in fat mass, yielding lower nadir body weights in male LPO. Given similarly accelerated growth rates after restoration of normal diet; males catch up to NPO control weight later than females. Juvenile LPO at 3-4 mo (≅ 7-10 y/o children in relative life span) exhibit what may be stress-dependent hypertension, apparent when confined in a sling and on first exposure to metabolic cage conditions, but fully normalizing over 4-6 days, perhaps as LPO animals acclimatized to monitoring cages. At least in males, neither obesity nor excess fat-free body mass is required for this early, largely systolic hyperreactivity to stress. **Conclusions** in females await additional studies. Temporal relationships are consistent with the hypothesis that accelerated growth, when superimposed on reduced nephron number, may contribute to development of hypertension despite normal body composition. Future studies will prevent catch-up growth to determine whether achieving excess body mass relative to nephron endowment is in fact essential to initiation of stress-dependent hypertension in juveniles and/or to hypertension in adults.

P3-041

N-Acetylcysteine Treatment and Recovery of the Left Ventricular Pressure (LVP) Function of the Adult Rat Heart Following Myocardial Ischaemia Reperfusion (IR): Effect of Prenatal Programming. Matthew J. Elmes, David Gardner[1], and Simon C. Langley-Evans, Division of Nutritional Sciences, School of [1]Human Development, University of Nottingham, Loughborough, Leicestershire, LE12 5RD, UK

Background We have previously shown that the LVP function of the isolated adult rat heart is significantly reduced following 30 minutes IR. Fetal undernutrition had no effect on LVP function in adult female hearts but hearts from male rats exposed to a low protein diet *in utero* exhibited greater cardiac dysfunction than was noted in controls. Free radicals produced upon reperfusion are implicated in the development of contractile dysfunction. The antioxidant glutathione has been proposed to play a key role in protection against free radical IR injury. Prenatal protein restriction modulates glutathione concentrations and metabolism in many tissues. Antioxidant capacity can be improved with N-acetylcysteine (NAC) treatment. The experimental aim was to assess whether NAC treatment could protect the LVP function from 30 minutes IR. **Methods** Pregnant Wistar rats were fed either a control (CON) 180 g casein/kg or low protein (LP) 90 g casein/kg diet throughout pregnancy with water available *ad libitum*. At birth litters were weaned onto standard laboratory chow (200g protein/kg). At 4 weeks of age systolic blood pressure was determined by tail cuff plethsmography. At 6 months of age female rats received 12.5 mg NAC i,p twice daily for 48 hours. Post NAC treatment, rats were anaesthetised by 3% isofluorane in 2L min^{-1} O_2 and killed by cervical dislocation. Hearts were rapidly excised, cannulated via the aorta to Langendorff perfusion apparatus, and perfused at a constant pressure with oxygenated Krebs Henseleit buffer in a coronary retrograde fashion at 37°C. A latex balloon was inserted into the left ventricle (LV) and adjusted to an end diastolic pressure of 5-8 mmHg. LV and perfusion pressure were constantly measured and recorded. All hearts were subjected to 30 minutes baseline recording prior to 30 minutes ischaemia and 60 minutes reperfusion. **Results**: Analysis of baseline LVP data following NAC treatment showed a significant difference in baseline function between CON (n=4) and LP (n=5) offspring. Baseline LVP was 2 fold higher in females exposed to the LP diet (33.21 ± 0.25 mmHg) when compared to CON (17.9 ± 0.06, P<0.004). Following 30 minutes ischaemia, LVP function was significantly (P<0.05) decreased relative to baseline in both dietary groups. However, the fall in LVP in LP fed rats, was no lower than the baseline function seen in CON fed offspring. **Conclusion**: 48 hrs pre-treatment with NAC has opposing effects on the LVP function of the female heart in CON and LP offspring. Protein restricted animals treated with NAC had higher baseline LVP function and improved recovery following IR. In contrast baseline LVP function was significantly lower and recovery following IR inhibited in CON animals treated with NAC. It is evident that NAC can protect the cardiac function of the heart in prenatally protein-restricted females that suffer an ischaemic event, but appears to be detrimental to rats exposed to a nutritionally adequate diet *in utero*.

P3-042

Prenatally Programmed Hypertension and its Effects on the Left Ventricular Pressure (LVP) Function of the Rat Heart Following Ischemia Reperfusion Matthew J. Elmes, David Gardner[1], and Simon C. Langley-Evans, Division of Nutritional Sciences, [1]School of Human Development, University of Nottingham, Loughborough, Leicestershire, LE12 5RD, UK

Background: Hypertension is a major risk factor for ischaemic heart disease (IHD). An age specific increase of 20(10) mmHg systolic(diastolic) blood pressure above average doubles the risk of IHD and represents a common cause of death among western populations over the age of 50. Fetal undernutrition is a well-established risk factor for hypertension and rats subject to protein restriction *in utero* have elevated systolic blood pressure in adult life. Little is known about the impact of prenatal undernutrition upon cardiac function. The aim of the present study was to investigate whether rats with programmed hypertension are more susceptible to ischemia reperfusion injury. **Methods**: Pregnant Wistar rats were fed either a control (CON) 180 g casein/kg or low protein (LP) 90 g casein/kg diet throughout pregnancy with water available *ad libitum*. At birth litters were weaned onto standard laboratory chow (200g protein/kg). At 4 weeks of age systolic blood pressure was determined by tail cuff plethsmography. At 6 months of age rats were anaesthetised by 3% isofluorane in 2L min^{-1} O_2 and killed by cervical dislocation. Hearts were rapidly excised, cannulated via the aorta to Langendorff perfusion apparatus, and perfused at a constant pressure (Palmer *et al*, 2004) with oxygenated Krebs Henseleit buffer in a coronary retrograde fashion at 37°C. A latex balloon was inserted into the left ventricle (LV) and adjusted to an end diastolic pressure of 5-8 mmHg. LV and perfusion pressure were constantly measured and recorded. All hearts were subjected to 30 minutes baseline recording prior to 30 minutes ischemia and 60 minutes reperfusion. **Results** At baseline LVP data was influenced by the interaction of sex and dietary treatment but there were no significant differences between CON and LP exposed male and female rats. Ischemia reperfusion decreased LVP function in the hearts of both sexes. The effect was similar between female CON (n=7) and LP (n=6) groups but in males LVP function was decreased in the LP (n=7) group when compared to controls (n=7), (P=0.057).

Maternal Dietary Group	Sex	Baseline LVP mmHg	LVP 60ins mmHg	% recovery LVP
CON	M	27.4 ± 4.1	15.4 ± 3.0	60.5 ± 12.6
CON	F	30.4 ± 3.5	10.6 ± 2.3	39.4 ± 9.4
LP	M	34.6 ± 3.9	11.7 ± 2.7	34.0 ± 7.6
LP	F	23.9 ± 4.7	9.9 ± 2.5	37.7 ± 9.0

Conclusion: In conclusion, exposure to a restricted protein intake during fetal life had a sex specific effect on the LVP function of the adult rat heart. The LVP function post ischemia was significantly higher in CON fed males than those on a LP diet, but this dietary effect was absent in female rats. It was evident that the recovery in LVP function following reperfusion ischemia in male offspring was impaired by maternal protein restriction during pregnancy. As a result we conclude that male hearts may be predisposed to cardiac dysfunction after such insults.

P3-043

Arterial Pressure and Renal Function Following Life-Long Exposure to a Low Protein Environment in Rats Chantal C. Hoppe[1], Roger G. Evans[2], Karen M. Moritz[1], John F. Bertram[1]; [1]Department of Anatomy and Cell Biology and [2]Department of Physiology, Monash University, Clayton, 3800, Australia.

Background: Limitation of maternal nutrient supply is known to alter fetal development and confer a greater risk of adult disease. A low protein diet administered to rats solely *in utero* is commonly associated with nephron deficit and elevated arterial pressure. The nephron deficit in this model has been proposed to play a major role in the associated hypertension and decline in renal function. This is based on the concept that a reduced glomerular filtration surface area limits urinary sodium excretion, leading ultimately to elevated arterial pressure. In many parts of the developing world, protein deficiency is a life-long condition. Yet surprisingly, animal models of life-long protein restriction have been little studied. Therefore, in the present study, the effects of a continuous low protein diet (throughout pregnancy and continued after birth) on adult arterial pressure and renal function were determined. The ability of these rats to handle a sodium load was also determined. **Methods:** Sprague-Dawley rats were born to mothers fed a low (8%) or normal (20%) isocaloric protein diet and maintained on this diet all their life. Rats that were born to mothers fed a low or normal protein diet were additionally fed a high (8%) salt diet at weaning. At an average age of 135 days, mean arterial pressure (MAP), heart rate (HR), renal plasma flow (RPF) and glomerular filtration rate (GFR) were determined in male offspring. **Results:** MAP was significantly less in those animals exposed to life-long protein restriction compared to normal rats (120±2 versus 128±2 mmHg, respectively, $P<0.05$). A high salt diet did not significantly affect MAP, but had significant effects on renal function. A high salt diet caused a 4-fold increase in GFR in protein restricted rats, but only a 43% increase in GFR in rats on a normal protein diet. RPF was not significantly altered by the high salt diet in either protein-restricted or protein-replete rats. **Conclusion:** Life-long protein restriction under these experimental conditions does not lead to hypertension, even with the added insult of increased salt intake. However, hyperfiltration in protein restricted rats on a high salt diet could potentially lead to renal pathology in later life. This remains to be determined.

P3-044

Offspring of Rats Fed Different High Fat Diets During Pregnancy Show Significant Difference in Blood Pressure and Vascular Function at 180 Days of Age Runa I Jensen, Joaquim Pombo, James A Armitage, Lucilla Poston and Paul D Taylor; Maternal and Fetal Research Unit, Division of Reproductive Health, Endocrinology and Development, Kings' College London, London SE1 7EH, United Kingdom.

Background: Previous data from our laboratory have shown an increase in blood pressure in female offspring of lard fed dams (rich in saturated fatty acids and monounsaturated fatty acids) at 180 days and 1 year of age. This study investigated the specific effects of 3 different maternal fat rich diets on adult offspring cardiovascular function. **Methods:** Sprague Dawley rats were fed *ad libitum* a control breeding diet (5% w/w corn oil), or a control diet supplemented with 20% w/w, saturated fat (palm oil, SFA), monounsaturated fat (rapeseed oil, MUFA) or polyunsaturated fat (corn oil, PUFA) 10 days before mating and throughout pregnancy and suckling. Within 48 hours of birth, litters were culled to 4 ♀ and 4 ♀ animals and weaned onto a control diet at 21 days (3.5% w/w corn oil). At six months of age blood pressure was measured continuously by radiotelemetry for 1 week following recovery from surgery (DSI, USA). Animals were humanely killed and third order branches of mesenteric arteries were dissected and mounted on a Mulvany-Halpern small vessel myograph (DMT, Denmark) and subjected to a standard normalisation and run-up procedure. Constrictor function was assessed to noradrenaline, and phenylephrine. Following preconstruction with noradrenaline, dilator function was assessed to acetylcholine, isoprenaline, and nitric oxide. Data were analysed by ANOVA incorporating diet and gender as independent variables. **Results**: Table 1 shows the mean ± SEM of 12-hour night-time averages over seven days for activity (arbitary/units), heart rate (beats/min), diastolic blood pressure (DBP, mmHg), and systolic blood pressure (SBP, mmHg) in female offspring at 180 days of age. Although there was a trend for raised SBP in the SFA and MUFA offspring, there was no significant difference in any of the parameters relative to offspring of controls However there was a significant difference in SBP between offspring of PUFA fed dams and offspring of SFA fed dams ($P<0.019$). No significant difference was observed in the male offspring.

	MATERNAL DIET			
	Control (n=9)	SFA (n=9)	MUFA (n=10)	PUFA (n=10)
Activity	3.5 ± 0.2	3.2 ± 0.3	2.7 ± 0.2	2.5 ± 0.2
HR	422.1 ± 8.2	422.4 ± 4.0	421.6 ± 5.1	407.7 ± 7.3
DBP	92.6 ± 2.0	91.7 ± 2.9	91.9 ± 0.6	93.8 ± 2.0
SBP	118.9 ± 2.9	127.2 ± 2.9 *	125.5 ± 4.3	116.3 ± 2.9 *

Within any row of the table, subscripted symbols denote significant differences (*$P<0.05$). There was no significant difference in vascular function in any of the groups relative to controls for any of the constrictors and dilators tested. **Conclusions**: In this study a high fat diet during pregnancy influences blood pressure but not vascular function in 180 day old offspring. These data indicate that a high content of SFA, compared with high PUFA, in the maternal diet programmes elevated blood pressure in female offspring. We have previously shown more marked hypertension in this fat feeding model at one year of age and this time point is currently being studied. If these data can be extrapolated to humans they will provide information translating to the dietary advice given in human pregnancy.

P3-045

Maternal Folic Acid Supplementation: Postnatal Nutrition Influences Brain Fatty Acid Levels in Adult Wistar Rats Sadhana Joshi*, Shobha Rao, Anvita Kale*, Mahabaleshwar Hegde* & Sahebarao Mahadik** * Interactive Research School for Health Affairs, Bharati Vidyapeeth, Pune 411043, Dept. of Biometry and Nutrition Unit, Agharkar Research Institute, Pune 411004, ** Dept of Psychiatry, Medical College of Georgia, Augusta, USA.

Background: Folic acid and LC-PUFA are known to have independent effects on fetal growth. However, our earlier study indicated that maternal folic acid supplementation (8 mg/kg diet) at marginal protein levels (12%) alters LC-PUFA metabolism in the brain of the offspring. These results were observed when the offspring were shifted to 18% protein diet. This renders some support to the fetal programming hypothesis. Since diets in poor communities are often inadequate in energy and protein, the present study examined the same level of folic acid supplementation with the offspring continuing on a 12% protein in the postnatal period **Methods**: During gestation female rats in 3 groups (n=6 in each) were fed casein diets with 18 g protein /100 g diet with 2mg folic acid / kg (CONT), 12g protein / 100g diet with 2mg folic acid /kg (Group A), 12g protein /100g diet supplemented with 8 mg folic acid/kg (Group B). Pups from CONT group were weaned on 18% diet while pups from Group A and Group B were weaned on 12 g protein/100g diet. Both male and female offspring from all the groups were studied for vital organs and brain fatty acid profiles at 6 mo. **Results**: There was no difference in total weight gain during pregnancy between groups. Litter weight at birth in Group A (74.7 ± 5.48g) was significantly higher ($p<0.05$) than control (65.3 ± 11.7g). However there was no difference in litter size and litter weights during lactation. Relative brain weights in male offspring from Group A (0.49 ± 0.09) was higher ($p<0.05$) than control (0.42 ± 0.03) indicating brain sparing. In contrast, female offspring from Group B (0.70 ± 0.08) showed higher ($p<0.05$) relative brain weight as compared to control (0.64 ± 0.07). Further higher ($p<0.05$) relative liver weights were seen in both Group A and Group B as compared to control. These changes were not observed in weights of organs when the offspring were shifted to a control diet in the earlier protocol indicating that poor postnatal nutrition can affect the size of vital organs. Brain fatty acid profile in males showed a significant reduction in both DHA (10.43 ± 2.38 Vs 13.75 ± 0.89 mg/100g fatty acid) and AA levels (8.74 ± 1.45 Vs 10.88 ± 0.56 mg/100 g fatty acid) in Group B as compared to CONT group. However these changes were not observed in females. The changes observed in brain fatty acid composition at 6 mo in this study was similar to that observed at 11 mo when the offspring were shifted to a 18% protein (control) diet in our earlier study. **Conclusion**: Folic acid supplementation at marginal maternal protein levels alters the brain fatty acid profile in spite of the fact that protein level continued to be marginal during lactation for dams and also for pups. In fact, the adverse effects of folic acid supplementation was seen at a much earlier age than when the offspring were shifted to a control diet. This study indicates that postnatal nutrition clearly plays an important role in determining the age of onset of adverse changes in brain fatty acid profile.

P3-046

Effect of Maternal Nutrition on Post-Weaning Body Composition in the Kitten RL Kelley, and AJ Lepine; Research & Development, Procter and Gamble Pet Health and Nutrition, Lewisburg, OH 45338

Background: There is a growing body of evidence that maternal malnutrition results in developmental changes that can have long-term health consequences in the offspring. The vast majority of studies conducted in non-human species have utilized known deficiencies, such as low-protein, to elicit developmental alterations, "Fetal Programming". However, malnutrition is simply a state of sub-optimal nutrition which can be the result of numerous factors. The following report is offered as serendipitous findings from a study that was conducted to determine the effect of maternal diet on reproductive performance in the queen and the growth, health status and development of the kitten and offers additional support on the importance of proper maternal nutrition. **Methods**: Sixteen queens were randomly assigned to 2 dietary treatment (TRT) groups (8/TRT) and exclusively fed (without restrictions) their assigned diet throughout the maternal phase (Breeding – 3 wks Post-Weaning). All queens had proven histories of being capable queens as evident by producing 1-2 prior litters without incident. In addition, all queens received full physical exams and were deemed healthy prior to study enrollment. Treatment diets were both commercially available kitten formulations that were considered "Complete & Balanced" and met or exceeded AAFCO guidelines for Growth & Reproduction. Both diets contained approximately 36% protein, while TRT A contained a higher level of fat (22 vs 15%) and subsequently a lower carbohydrate fraction (26 vs 32%) compared to TRT B. Fifty-One kittens (31-A and 20-B) were selected from the 16 litters at weaning and assigned to one of the 2 TRT diets. All selected kittens received a physical exam and deemed normal and healthy prior to enrollment. Approximately

½ of the kittens from each maternal TRT group were retained on their respective maternal diet (MD) and the remainder switched to the opposing TRT diet throughout the juvenile phase (weaning – 24 weeks of age). Each group was balanced as much as possible for weaning weight and gender. All kittens were monitored throughout the study for diet intake, body weight (BW) and nutrient profiles every 2 wks. Body composition (BC) estimates, at 24 wks, were obtained via dual-energy x-ray absorptiometry (DEXA) using a Hologic QDR-2000 plus X-Ray Bone Densitometer with pediatric software. All statistical comparisons were made using the GLM procedure of SAS. **Results**: All kittens were found to have similar intakes (kcals/kg BW) throughout the juvenile phase. As one would expect, absolute intake was less in the more caloric dense TRT A group. Kitten BW was similar at weaning (TRTA @ 801g vs TRTB @ 799g) with no differences observed through 14 weeks of age for either MD or post-weaning diet (KD). However, from 16 wks forward, significant separation in kitten BW was observed for both MD and KD, with KD having the greatest effect on final BW ($P<0.02$). Despite the effect on BW, KD was found to have little influence on BC. In contrast, MD was found to significantly influence kitten BC. Kittens reared by queens fed TRT A were found to possess significantly ($P<0.01$) lower body fat (26% vs 32%) and higher lean tissue (71% vs 66%) regardless of their post-weaning diet. **Conclusions**: While additional studies will be required to determine the physiological mechanism, findings from this preliminary study strongly support the importance of maternal nutrition regarding the long-term health of offspring. Most importantly, these findings suggest that even a diet without deficiencies, or at least a known deficiency, may induce developmental changes altering the susceptibility to certain long-term health risks, such as obesity, in the offspring. **Key Words**: Maternal Nutrition, Cat, Obesity, Fetal Programming

P3-047

Vascular Structural Changes in Hypertensive Offspring of Nutrient Restricted Pregnant Rats Omid Khorram, Mazdak Momeni, Mina Desai, Michael Ross Department of OB/Gyn, Harbor-UCLA Medical Center, Torrance, CA 90502, USA

Background: Human and animal studies have demonstrated the gestational programming of offspring hypertension and obesity. Although induced by prenatal interventions of maternal undernutrition or administration of glucocorticoids, the mechanism(s) involved in the development of offspring hypertension has not been elucidated. As most studies have described the vascular phenotype *following* the development of hypertension, we sought to identify prenatal and neonatal vascular factors that predispose to hypertension. We hypothesized that prenatal stress alters the expression of angiogenic/vasculogenic genes resulting in altered newborn vascular structure which culminates in hypertension during postnatal life. **Methods:** Pregnant Sprague Dawley rats and offspring were studied. Control dams received ad libitum food, whereas study dams were 50% nutrient-restricted (NR) NRom pregnancy day 10 to 21 to produce growth restricted newborns. At birth, litter size was culled to 4 males and 4 females. All pups were nursed by dams fed ad libitum and were weaned at 3 weeks to ad libitum feed. Offspring were sacrificed at 1 day and 2 months of age. The abdominal aorta was sectioned, stained for elastin (Van Giessen), collagen (Sirius Red), and mucopolysaccharides (colloidal iron), and examined histologically. Image analysis was performed using Image Pro software. Mesenteric blood vessels (proximal colon) were sampled and number of vascular brances quantified. Arterial blood pressure was measured in offspring at 2 months of age. **Results:** By 2 months of age both genders had a significant increase in mean arterial blood pressure compared to the controls. Aortas from 1 day old NR rats showed a significant increase in the relative amount of elastin and significantly lower mucopolysacharide and collagen content compared to control animals. However, at 2 months of age, the collagen content of NR aortas was significant increased, and the difference in elastin content between the two groups was no longer present. In the proximal colon, mesenteric vascular branches were significant reduced in the NR as compared to control animals. **Conclusion:** At birth the composition of the aorta in the NR animal is consistent with a more compliant vessel. This compensatory mechanism fails with time such that the vessel becomes structurally less compliant as indicated by increased extracellular matrix collagen deposition. The decrease in mesenteric vascular branching in the NR neonates provides an additional vascular mechanism for hypertension, namely increased resistance to blood flow. These findings are the first to provide a structural basis at the level of vasculature for development of hypertension in NR offspring.

P3-048

Vascular Expression of Angiogenic Proteins in Offspring of Undernourished Rats Omid Khorram, Guong Han, Mazdak Momeni, Mina Desai, Michael Ross Department of Ob/Gyn, Harbor-UCLA Medical Center, Torrance, CA 90502, USA

Background: Maternal undernutrition results in offspring that develop hypertension, obesity and diabetes as adults. Our interest has been in elucidating the prenatal/neonatal processes that predispose the animal to hypertension, with a focus on the vascular system as a target of programming. We have hypothesized that prenatal nutritional stress alters the expression of angiogenic/vasculogenic genes in vascular beds which result in offspring with altered vascular structure. This altered vascular anatomy may increase the susceptibility of the vessels to shear stress resulting in endothelial dysfunction and eventually hypertension. **Methods:** Pregnant Sprague-Dawley rats had 50% food restriction from day 10 of gestation until delivery. The offspring were sacrificed at 1 day of life. The abdominal aorta and liver were dissected and flash frozen in liquid nitrogen. After protein extraction samples were subjected to western blot analysis for angiogenic proteins. Mean band densities were compared by Student's t-test. **Results:** There was a 2 fold greater expression of C-reactive protein (CRP) in the aorta of control animals as compared to FR offspring. In contrast, in the liver an opposite pattern was found with significantly greater expression of CRP in the liver of FR animals as compared to the controls. Unexpectedly, the expression of eNOS was two fold higher in the aorta of FR animals as compared to controls. The expression of two other angiogenic proteins, namely VEGF and COX-2, were significantly lower in the aorta of FR offspring as compared to controls. **Conclusions:** Marked changes in the expression of angiogenic proteins occur in the aorta. Our data demonstrates that in FR offspring liver inflammation occurs early in life, whereas the aorta demonstrates compensatory adaptive changes which prevent the initiation of inflammatory process. We hypothesize that lower vascular CRP results in higher eNOS expression in the aorta. Greater NO will maintain relaxation of the vessel and decrease elastase activity, as previously demonstrated resulting in greater vessel compliance. The decreased expression of VEGF/COX-2 in the aorta may be a generalized reflection of reduced expression of these angiogenic factors in other vascular beds, and may explain the decreased mesenteric vascular branching in the FR neonatal offspring.

P3-049

Pre-partum Nutrition Programs Energy Metabolism of Offspring in Later Life in Sheep Kiani, A., Chwalibog, A., Nielsen, M.O., and Tauson, A. H., Department of Animal and Veterinary Basic Sciences, The Royal Veterinary and Agricultural University, Gronnegaardsvej 7, DK-1870 Ferderiksberg C, Denmark

Background Nutritional metabolic programming indicates the possibility that the quantity and quality of dietary nutrients consumed prepartum and immediately postpartum period may permanently affect offsprings subsequent metabolism(1). Nutritional factors not only have short-term effects on growth, body composition and body function, but also have long-term effects on body characteristics(4). However, the nature of the long-term effects on energy metabolism in later life has yet to be known. **Methods** Adult twin-pregnant offspring born to dams fed either *ad libitum* (Control) or 60 % of their requirements (maternal restricted)(MR) during the last trimester of gestation (~147 days) were fed restricted diets (60% of requirements) from day 42 *pre-partum* until term during their second pregnancy. Body weights, body condition score (BCS) and blood samples were collected at 49, 28, and 10 days prior to parturition. Non-fasting plasma glucose concentration, insulin, β-hydroxybutyrate(BOHB) and non-esterified fatty acid (NEFA) were measured. At day 137 of gestation, O_2 consumption and CO_2 production was measured by means of open-air-circuit respiration unit. Heat energy (HE) and respiratory quotient (RQ) were calculated from gaseous exchange (CO_2, O_2, CH_4) and nitrogen excretion in urine (UN) using Brouwer equation (2). Data were analysed as repeated-measures by the MIXED procedure in SAS® (3) using a model with maternal nutrition as the fixed effect, animal as the random effect, and day as the repeated measure. **Result** Table1. Effect of maternal nutrition on body weight, plasma metabbolite, insulin concentration and energy metabolism in later life in sheep

Treatment	Control(n=5)			Maternal Restricted(n=5)				*P*-value	
Days *Pre-partum*	49	28	10	49	28	10	SEM[3]	Treatment	Day
Body weight (kg)	89.7[ad]	85.3[b]	83.1[c]	75.2[e]	73.2[g]	72.6[g]	2.7	0.01	<.0001
BCS	4.5[a]	4.2[ab]	4.0[bc]	4.2[ab]	3.8[c]	3.5[d]	0.1	0.07	<.0001
Glucose (mmol/l)	3.1[a]	2.5[bc]	2.8[ab]	2.5[bc]	2.0[d]	2.5[bc]	0.2	0.01	<.0.01
Insulin(µg/l)	0.30[ab]	0.15[cd]	0.11[cd]	0.31[a]	0.16[c]	0.09[d]	0.04	0.98	<.0001
Insulin/Glucose	0.09[ab]	0.07[bc]	0.04[c]	0.13[a]	0.08[bc]	0.04[c]	0.02	0.39	<.0001
NEFA(mmol/l)	0.4[cd]	0.8[bc]	1.3[a]	0.3[d]	0.7[c]	1.1[ab]	0.14	0.26	<.0001
BOHB(mmol/l)	0.7[bc]	0.9[b]	1.3[a]	0.5[c]	0.7[bc]	0.8[b]	0.1	0.01	<.001
BOHB/Glucose	0.24[bc]	0.36[a]	0.44[a]	0.21[c]	0.38[ab]	0.31[a]	0.04	0.22	<.001
RQ[1]	-	-	0.83[b]	-	-	0.88[a]	0.01	0.05	-
HE/ MBW[2] (KJ/day)	-	-	451[b]	-	-	504[a]	14	0.03	-
Urinary nitrogen(g/d)	-	-	7.2	-	-	6.8	0.4	0.44	-

[1]RQ=CO_2 production/O_2 consumption, [2]Metabolic Body Weight($BW^{0.75}$) [3]Standard error of means [4]Values with different supercript in the same row are significantly different($P<0.05$) **Conclusion** Non-fasting plasma glucose concentration was decreased in MR offspring, suggesting that glucose tolerance had deteriorated in this group. The lower plasma BOHB and the higher RQ in MR indicate the less oxidation of fat. Furthermore, maternal restriction caused an increase in HE/MBW in the adult offspring of MR group which means losing the potential energy that could be used to adapt to the increased demands of pregnancy. **Refrences** 1. Barker DJP. 1994. Mothers, babies and disease in later life. London: BMJ Publishing Group 2. Brouwer E. 1965. Report of sub-committee on constants and factors. In *Energy Metabolism of Farm Animals*, ed. KL Blaxter,441-443. Academic Press, London: 3. Littel RC, Henry PR, Ammerman CB. 1998. Statistic analysis of repeated measures data using SAS procedures *Journal of Animal Science* 76:1216-31 4. Lucas A. 1998. Programming by early nutrition: An experimental approach *Journal of Nutrition* 128:401S-6S

P3-050

Maternal and Fetal Micronutrient Status in Rapidly Growing Pregnant Adolescent Sheep

J.S. Luther[1,2], R.P. Aitken[1], J.S. Milne[1], H.J. McArdle[1], L. Gambling[1], D.A. Redmer[2], L.P. Reynolds[2] and J.M. Wallace[1]. [1]Rowett Research Institute, Aberdeen, AB21 9SB, UK. [2]North Dakota State University, Fargo, North Dakota, 58105, USA.

Background: Inadequate micronutrient status has variously been associated with poor pregnancy outcome. Pregnant growing adolescents are potentially vulnerable to micronutrient deficiencies, as competition exists between growth of the mother and her developing fetus. The aim of the current study was to determine maternal and fetal liver Fe, Cu and Zn status in an adolescent sheep model characterized by rapid maternal growth, premature delivery and fetal growth restriction. **Methods**: Singleton pregnancies to a single sire were established by embryo transfer in adolescent ewes of similar age, weight and adiposity. Thereafter, dams were offered a control (C, n=16) or high (H, n=12) amount of a complete diet to maintain adiposity or promote rapid maternal growth, respectively. Autopsies were performed on either day 90 or 130 of gestation (term=145 days). Maternal and fetal liver micronutrient concentrations were determined by graphite furnace atomic spectrophotometry. **Results**: Maternal empty body and liver mass, and maternal blood volume increased as gestation advanced and these parameters were further elevated in H dams (Table). An identical but separate study associated this H-dietary level with increased (P<0.001) maternal haematocrit and total haemoglobin at day 130 of gestation (H: 39.8 ± 0.80 and 12.3 ± 0.15 versus C: 33.8 ± 0.68 % and 10.5 ± 0.18 g/dL, respectively). Maternal Fe decreased (P<0.02) as gestation advanced, and was further reduced (P<0.001) in the rapidly growing H-group. In contrast, maternal Zn increased (P<0.001) from day 90 to 130 of gestation, and both maternal Zn and Cu reflected maternal dietary intake (Table). Placentome mass was reduced in the H group and by late gestation resulted in marked fetal growth restriction (Table). Fetal Cu, but not Fe or Zn status, reflected maternal intake, and, irrespective of maternal intake fetal Cu was negatively correlated with fetal mass (r=-0.55, P<0.05) at day 130 of gestation. No other significant associations were detected between maternal or fetal liver micronutrient status and fetal weight.

Table. Maternal and fetal liver micronutrient status during adolescent sheep pregnancies

	Day 90		Day 130					
	Control	High	Control	High	Pooled SE	Nutrition	Day of Gestation	Interaction
Maternal body mass (kg)	28.0	42.0	31.4	49.2	1.91	P<0.001	P<0.001	ns
Blood volume (ml)	1709	2355	2313	2544	17.8	P<0.001	P<0.003	ns
Liver mass (g)	602	1186	725	1310	11.2	P<0.001	P<0.02	ns
Fe (μg/g)	140.2	46.8	83.5	55.1	5.06	P<0.001	P<0.02	P<0.003
Cu (μg/g)	11.3	37.5	6.0	23.8	3.94	P<0.001	ns	ns
Zn (μg/g)	69.5	76.8	79.3	85.5	2.45	P<0.007	P<0.001	ns
Placentome mass (g)	566	480	557	301	21.5	P<0.006	ns	ns
Fetal mass (g)	653	732	4518	3603	12.2	P<0.03	P<0.001	P<0.009
Liver mass (g)	43.6	47.1	135.9	110.1	4.83	ns	P<0.001	ns
Fe (μg/g)	586.9	434.5	224.5	253.3	17.6	ns	P<0.03	ns
Cu (μg/g)	6.5	9.4	9.3	24.6	2.19	P<0.001	P<0.001	P<0.002
Zn (μg/g)	42.6	57.3	74.3	57.9	4.60	ns	P<0.06	P<0.07

Conclusion: The reduced maternal Fe stores in the overnourished adolescent dams may reflect increased Fe requirements for maternal growth and associated expansion of blood volume. The increases in maternal Zn and Cu stores simply reflect dietary intake and in both groups almost certainly exceed minimum maternal and fetal requirements. In this paradigm, alterations in maternal micronutrient status are secondary to maternal intake and the associated placental growth restriction, and, do not appear to regulate fetal growth. *Funded by SEERAD*

P3-051

Protein and Fatty Acid Composition of the Maternal Diet Impacts on the Developing Insulin Axis in the Rat

C.A. Maloney, A Czopek, C Lilley, S.M. Hay and W.D. Rees. Rowett Research Institute, Bucksburn, Aberdeen. AB21 9SB Scotland UK.

Background: The disorders that comprise metabolic syndrome are complex ailments that develop gradually throughout life as the homeostatic mechanisms regulating glucose, amino acid and lipid metabolism fail to cope with the prevailing nutritional environment. The available evidence suggests that metabolic control mechanisms are established (programmed) early in life. Thus, inappropriate programming of metabolic regulation may initiate or accelerate the development of metabolic syndrome. Our previous studies have shown that dams fed Hope farms (HF) control diet (20% protein, soya oil) give birth to offspring that have an altered insulin response to a glucose load at 20 weeks of age when compared with offspring from dams fed HF low protein (8%) diet, or either of two University of Southampton diets (18% & 9% protein, corn oil). All four of these diets differ in both the relative amounts, and types of carbohydrate and lipid used (e.g. sucrose vs dextrose). This study investigated if the type of oil (corn vs soya) fed to pregnant rats is responsible for altering the response of the offspring to glucose load later in life. **Methods:** Female Rowett Hooded Lister rats were fed for 2 weeks semi-synthetic diets containing either 180g/kg casein with either corn oil (18C) or soya oil (18S), 90g/kg casein with either corn oil (9C) or soya oil (9S) with the oil content being 7% of the diets. The females were then mated with normal males and continued to be fed the experimental diets until birth. During lactation the dams received stock diet and all offspring remained on stock diet until 25 weeks of age at which time they (a male and a female from each dam) underwent an oral glucose tolerance test after which they were killed and tissues were harvested for analysis. Gene expression was determined by real time RT-PCR. **Results:** At 25 weeks of age the female offspring differed in their response to an oral glucose load. The 18S group had a raised insulin release when compared to both the 18C and 9C offspring. This was due to maternal dietary oil rather than protein (p<0.03) and occurred despite the groups having the same plasma glucose response. These observations were not seen in the male offspring. The insulin stimulated gene expression revealed that the 9S group had increased ACC (Acetyl-CoA Carboxylase) and CPT-1 (Carnitine Palmitoyl Transferase) expression. Two way ANOVA analysis revealed the difference in ACC-1 expression to be due to an oil x protein interaction (p<0.004) while the CPT-1 difference was due solely to protein (p<0.03). **Table1: Female offspring characteristics at 25 weeks of age** (averages ± sem). All data are arbitrary units. iAUC: incremental area under curve. Values in bracket describe rats per group. Numbers not sharing the same superscript in rows are significantly different (p<0.05)

Diet	18C (7)	9C (6)	18S (4)	9S (6)
Insulin iAUC	1634 ± 253 [a]	1667 ± 260 [a]	2705 ± 345 [b]	2097 ± 308 [a,b]
Glucose iAUC	201 ± 28 [a]	192 ± 21 [a]	235 ± 39 [a]	188 ± 22 [a]
ACC-1 gene expression	32.9 ± 1.9 [a]	30.0 ± 3.7 [a]	24.6 ± 3.4 [a]	41.4 ± 3.0 [b]
CPT-1 gene expression	33.5 ± 2.2 [a]	39.1 ± 5.3 [a,b]	33.5 ± 2.2 [a]	51.2 ± 5.7 [b]

Conclusions: The effects of two maternal diet macronutrients can interact to influence the development of the offspring. Soya oil has a higher content of α-linolenic acid than corn oil and this omega 3 fatty acid has been implicated in altering insulin sensitivity. The impact of reduced maternal protein on the expression of genes involved in fat metabolism was only seen in a diet with a higher α-linolenic acid content. Therefore, the protein content of the maternal diet may be important only when it interacts with the effects of other dietary components, alters fetal development and results in the aberrant programming of the offspring's metabolism. This work was supported by the European Union Fifth Framework programme NUTRIX (QLK1-2000-00083) and the Scottish Executive, Environment and Rural Affairs Department as part of the Rowett Research Institute core funding.

P3-052

Sex-specific Up-regulation of Renal Type 2 Angiotensin Receptor mRNA Expression in Hypertensive Rats Exposed to a Low Protein Diet or Carbenoxolone *in utero*.

Sarah M[c]Mullen and Simon C. Langley-Evans School of Biosciences, University of Nottingham, Loughborough, LE12 5RD.

Introduction.

The feeding of a low protein diet to pregnant rats has consistently been shown to program an increase in blood pressure in the offspring. Evidence suggests that the hypertensive effects are mediated by permanent alterations in renal structure and function, initiated by over-exposure of the fetus to maternal glucocorticoids. This study examined the effects of low-protein and glucocorticoid exposure on nephron number and mRNA expression of the type 2 angiotensin receptor (AT_2R), and the modulation of these effects by postnatal AT_1R inhibition. **Methods**. Pregnant Wistar rats were assigned to control (CON, 18 % casein, n=11), low-protein (MLP, 9% casein, n=10) and carbenoxolone (CBX, 18 % casein, n=12) treatments. CBX rats received daily injections of carbenoxolone (12.5mg/kg), an inhibitor of 11β-hydroxysteroid dehydrogenase (11β-HSD), for the last 7 days of pregnancy. CON and MLP rats received saline injections. At birth, litters were transferred to standard chow and culled to 8 pups. Between 2 and 4 weeks of age, a specific AT_1R inhibitor (L158-809) was added to the drinking water (25 mg/l) of half the litters from each group. Kidneys were collected at 4 and 20 weeks. Nephron number was counted by a maceration method. AT_2R mRNA expression was assessed by real-time RT-PCR and normalized to β-actin. Data was analysed by 4-way ANOVA to assess the main and interaction effects of prenatal and L158-809 treatments, age and sex. **Results**. There was a significant effect of prenatal treatment on nephron number (P<0.001), which was decreased in both MLP (39%) and CBX (35%) compared to CON. This effect interacted with L158-809 treatment (prenatal*L158-809, P<0.01), which reduced nephron number in CON offspring (32%). The effect of prenatal treatment on AT_2R expression (P<0.001, Table 1) interacted with age (P<0.01) and sex (P<0.01). Female MLP exhibited decreased AT_2R expression at 4 weeks followed by an increase at 20 weeks. Female CBX showed up-regulation of AT_2R at both 4 and 20 weeks. Postnatal L158-809 treatment prevented the MLP and CBX effects, exerting a down-regulatory effect on AT_2R mRNA expression overall (P<0.01). AT_2R mRNA expression was consistently lower in male offspring and not modulated by pre- or postnatal treatment, with the exception of a transient up-regulation in CBX males at 4 weeks.

Table 1. Relative AT_2R mRNA expression in CON, CBX and MLP offspring at 4 and 20 weeks (mean ± S.E.M).

Week	Sex	Control		MLP		CBX	
		Water	L158-809	Water	L158-809	Water	L158-809
4	M	0.076 ± 0.018	0.068 ± 0.024	0.065 ± 0.023	0.034 ± 0.009	0.134 ± 0.027	0.063 ± 0.014
	F	0.428 ± 0.137	0.276 ± 0.094	0.112 ± 0.073	0.452 ± 0.098	0.921 ± 0.333	0.425 ± 0.166
20	M	1.346 ± 0.341	0.682 ± 0.117	1.313 ± 0.085	1.479 ± 0.275	1.064 ± 0.274	1.237 ± 0.128
	F	2.032 ± 1.409	1.922 ± 0.139	11.161 ± 3.458	3.163 ± 0.303	9.296 ± 1.665	4.444 ± 1.082

Conclusions. Both MLP and CBX offspring exhibited reduced nephron number and up-regulation of AT_2R at 20 weeks. However, the transient decrease in AT_2R mRNA observed in 4 week MLP but not CBX offspring indicates that the precise mechanisms may differ. The reduced nephron number in CON offspring given L158-809 from 2 to 4 weeks of age demonstrates that the detrimental effect of postnatal AT_1R inhibition is not limited to the period of nephrogenesis, indicating a role for angiotensin II in post-nephrogenesis renal maturation and remodelling. AT_2R is known to be up-regulated in response to renal injury, a response which is thought to protect the kidney from the ongoing pathology. The lower basal expression and attenuation of such a response in male offspring may explain their increased susceptibility to and faster progression towards renal disease and hypertension.

P3-053

Effect of Periconceptional Undernutrition on Insulin Sensitivity at 65 and 120 days Gestation in Singleton Bearing Pregnant Ewes Jaquiery AL, Oliver MH, Rumball C, Buckley AJ, Harding JE Liggins Institute, Faculty of Medical and Health Sciences, University of Auckland, Private Bag 92019, Auckland New Zealand

Background Periconceptional undernutrition alters fetal growth, metabolism and endocrine status in late gestation. A possible mechanism underlying these effects could be nutritionally induced perturbation of the maternal physiological adaptations to pregnancy. Development of maternal insulin resistance is one such adaptation. **Aims** To determine the effects of periconceptional undernutrition in sheep on maternal insulin sensitivity in mid and late gestation. **Methods** Ewes were either: well nourished (N); undernourished from 61d before to 30d after mating (UN-60-30d); undernourished from 61d before until mating (UN-60-0d); undernourished from 2d before to 30d after mating (UN-2-30d). Well nourished ewes were fed to maintain body weight ±5%. Undernourished ewes were fed to achieve and maintain 15% weight loss after an initial 2d fast. At 65 and 120d of gestation, singleton-bearing ewes underwent hyperinsulinaemic-euglycaemic clamp (HEC). Insulin sensitivity was calculated as the ratio of steady-state glucose infusion rate (mg glucose/kg/min) to steady-state plasma insulin concentration (μU/ml). Groups were compared using factorial ANOVA with Fishers post-hoc correction for multiple comparisons.

Results

Insulin sensitivity (mg.l/μU/kg/min)	N pregnant	N Non-pregnant	UN-60-30d pregnant	UN-60-30 non-pregnant	UN-60-0d pregnant	UN-2-30d pregnant
65days	2.4±0.3 n=10	3.9±0.4* n=6	5.7±0.92† n=12	5.0±0.8 n=5	4.9±0.5† n=8	3.5±0.4 n=8
120 days	5.7±0.6 n=6		5.3±0.5 n=7		6.8±0.9 n=5	4.8±0.7 n=7

Values are mean±SE *$p<0.005$ for comparison between pregnant and non-pregnant animals in the same nutritional group † $p<0.03$ for comparison with N pregnant group Singleton bearing ewes undergoing prolonged undernutrition prior to conception failed to develop the pregnancy-related insulin resistance seen in normally nourished ewes at 65 days gestation, despite having been refed for 35-65d. Undernutrition for 30 days from the time of mating had no effect on maternal insulin sensitivity at 65 days. By late gestation, the differences in insulin sensitivity between nutritional groups had resolved. **Conclusions** Prolonged undernutrition before but not after mating inhibited the normal development of maternal insulin resistance in mid pregnancy. Impaired maternal physiological adaptation to pregnancy may be one mechanism by which periconceptional undernutrition alters later growth and development of the offspring.

P3-054

The Effects of Maternal Mild Protein Restriction on Stroke Incidence and Blood Pressure in Stroke-prone Spontaneously Hypertensive Rats Lila Otani, Tomohide Yasumatsu, Megumi Murakami, and Tetsuo Murakami ; Division of Advanced Life Sciences, Graduate School of Agriculture, Kinki University 3327-204 Nakamachi, Nara, 631-8505, Japan

Background : Maternal undernutrition and protein restriction during pregnancy might cause hypertension in offspring. We are focused on the influence of maternal protein restriction on development of offspring stroke incidence. This study investigated the effects of maternal protein restriction during pregnancy on offspring's blood pressure in stroke-prone spontaneously hypertensive rats (SHRSP), which are the animal models for human essential hypertension and stroke. **Methods :** Virgin female SHRSP were mated with littermate males of the same strain. From the day of conception, the females were fed a 20% casein diet (control) or a 9% casein diet (low protein) until the end of pregnancy. The SHRSP in the control and low-protein exposed groups were further divided at 10 weeks of age into a tap water group and a 1% saline solution group. All offspring were provided with a commercial diet *ad libitum*. Plasma aldosterone concentrations were determined at 12 weeks of age. **Results :** No differences were seen between the control group and low-protein exposed group in regard to body weight, blood pressure elevation, or life span. The plasma aldosterone concentrations of the low-protein exposed group (135.7 ± 7.6 pg/ml) ware similar to the control group (127.3 ± 8.8 pg/ml). On the other hand, a remarkable elevation of blood pressure in response to salt loading was seen in low-protein exposed group. The survival duration was significantly shorter in the low-protein exposed group (113 ± 4 days) than in the control group (135 ± 22 days; $p<0.05$). The plasma aldosterone concentrations of low-protein exposed group (116.5 ± 9.8 pg/ml; $p<0.05$) were significantly higher than the control group (97.9 ± 9.9 pg/ml). **Conclusion** : This study has shown that maternal protein restriction in SHRSP leads to salt sensitive hypertension and a shortened life span. Abnormality of aldosterone metabolism in response of salt loading was observed in low protein exposed SHRSP and this is considered to induce onset of stroke and a shortened life span.

P3-055

Dietary Folate, Methionine and Choline Deficiency Increases Plasma Homocysteine and Modifies Lipid Metabolism in the Dam, Changing Insulin in the Rat Fetus S.M. Hay, C.A. Maloney and W.D. Rees. Rowett Research Institute, Bucksburn, Aberdeen. AB21 9SB Scotland UK.

Green leafy vegetable intake in adulthood has been associated with reduced risk of diabetes and ischemic heart disease probably because they are a major source of folate, which is known to reduce plasma homocysteine levels. It has also been reported that diets containing adequate levels of green leafy vegetables are associated with increased birth weight, birth length, head circumference, placental weight and skin fold thickness. These observations suggest that folic acid plays a critical role in fetal development and may be an important factor in the fetal origins of adult disease. Folic acid is involved in a series of cyclical metabolic reactions, which also involve methionine and choline. Semi-synthetic diets based on casein contain high levels of methionine and choline, which may be protective and mask the effects of folate deficiency. Therefore in this study of folic acid deficiency in the pregnant rat, diets low in methionine and choline were also included. Female Rowett Hooded Lister rats were fed semi-synthetic diets containing the equivalent of 180 g/kg casein (Control), deficient in folic acid (-F), deficient in folic acid and low in methionine (-F LM), deficient in folic acid and low in choline (-F LC), and in combination (-F LM LC). After being fed the experimental diet for two weeks the animals were mated with normal males consuming stock diets. The dams continued to be fed the experimental diets until they were killed on day 21 of gestation. Table1 Maternal and fetal characteristics at necropsy

Diet	Control (n=8)	-F (n=6)	-F LM (n=5)	-F LC (n=6)	-F LM LC (n=7)
Litter size	14.5	13.6	13.6	12.3	13.1
Dam plasma homocysteine (nmol/ml)	4.5 ± 0.3 ^{a}	20.4 ± 3.1 ^{b}	23.9 ± 1.9 ^{bc}	33.9 ± 3.3 ^{d}	28.9 ± 2.4 ^{cd}
Mean fetal weight /litter (g)	4.2 ± 0.1 ^{ab}	4.9 ± 0.3 ^{c}	3.6 ± 0.1 ^{d}	4.3 ± 0.2 ^{a}	3.8 ± 0.2 ^{bd}
Dam hepatic triglyceride content (nmol/mg tissue)	8.66 ± 1.63 ^{a}	10.54 ± 3.16 ^{a}	13.67 ±1.06 ^{a}	9.53 ± 1.33 ^{a}	26.90 ± 3.77 ^{b}
Fetal pancreas insulin content (ng/mg tissue)	172.6 ± 3.6 ^{a}	230.7 ± 12.7 ^{b}	n.d.	n.d.	209.2 ± 3.9 ^{b}

Numbers not sharing the same superscript are significantly different ($p<0.05$) Folate deficiency significantly increases plasma homocysteine concentrations during gestation. Unexpectedly folate deficiency also led to an increase in fetal weight. Feeding a low methionine or a low choline diet ameliorated the effect on fetal weight. A combination of folate, methionine and choline deficiency induced a significant increase in the triglyceride content of the maternal liver. These results suggest that folate, methionine and choline in the maternal diet interact to change lipid metabolism in the dam. Folate deficiency in isolation or in combination with low methionine and choline increased the concentration of insulin in the fetal pancreas and this suggests that folate and choline deficiency influence the development of the fetal insulin axis. The changes in lipid metabolism suggest that diets low in folate and choline but high in fat may be especially harmful to the fetus. Interactions between folate, methionine and choline influence fetal development and may be involved in metabolic programming. This work was supported by the Scottish Executive, Environment and Rural Affairs Department as part of the Rowett Research Institute core funding.

P3-056

Disturbance of the Development of the Fetal Baboon Brain Resulting From Maternal Undernutrition is Associated With Systemic But not Local IGF Production M. Schwab[1], I Antonow-Schlorke[1], N. Schlabritz-Loutsevitch[2], K. Stuchlik[1], M.Levitz[4], L. Cox[3], T.J. McDonald[2], O.W. Witte[1], P. Nathanielsz[2]; [1]Dept. Neurol., Friedrich Schiller Univ. Jena, Germany; [2]Center for Pregnancy and Newborn Research, Dept. of ObGyn and [3]Foundation for Biomedical research University of Texas, San Antonio, TX, USA; [4]Dept. of ObGyn, New York University School of Medicine, NY, U.S.A.

Aim: We determined the effects of 30% global maternal nutrient restriction on insulin growth factors (IGF's) and early maturation of the fetal primate brain. Expression of IGF depends on the nutritional state in newborn rats. **Methods:** Pregnant baboons were either fed *ad libitum* (n=8) or 70% of the weight adjusted *ad libitum* diet (n=6). At 0.5 gestation fetuses were delivered by Cesarean section. We evaluated cell proliferation (anti-ki67) and apoptotic cell death (TUNEL), the protein expression of glial (GFAP for immature and S-100ß for mature astroglia) and neuronal cell lineages (DCX for immature and NeuN for mature neurons). In addition, neurite density (silver amplification) and myelination (levanol) was estimated. Specific labeling was semi-quantified using a computer-assisted image analyzing program. Circulating IGF-1 (by Immulite assay) and IGF-1 expressed in the brain was examined using real time RT-PCR and immunohistochemistry. **Results:** Control and nutrient restricted fetal brains did not differ in weight or gross anatomy at 0.5 gestation. The proliferative subventricular zone thickness was reduced to 56.6±11.5% after maternal nutrient restriction ($p<0.05$). Maternal nutrient restriction led to both an increase of the number proliferating cells by 42.5±7.9% and apoptotic cells by 118.9±97.6% in the subventricular zone. Therefore, the increase in the ratio of programmed cell death to cell proliferation rose by 76.4 % approaching significance ($p<0.1$). In the subventricular zone, fetuses of nutrient restricted mothers showed a reduction of S-100ß immunoreactivity by 65.6±19.6% ($p<0.05$). In contrast, GFAP immunoreactivity and the neuronal expression for DCX and NeuN did not change. Moreover, nutrient restriction led to a decline of neuronal arborization and myelination in the prospective neocortex by 55.5±23.6% and 41.0±29.6%, respectively ($p<0.05$). There was no change in mRNA for IGF-1 or –2 in the frontal cortex. At the this stage of development IGF-1 protein expression could not be demonstrated in the brain. Maternal nutrient restriction, however, decreased circulating IGF-1 that is produced in the liver ($p<0.05$). **Conclusion:** These observations indicate that moderate maternal nutrient restriction impairs brain maturation at the levels of cell proliferation, migration and development of the cortical neuronal network. These effects are at least in part mediated by suppression of circulating IGF-1.

P3-057

Programmed Deterioration of Renal Function is Exacerbated by a Disruption in the Glutathione Redox System D.V. Sculley and S.C. Langley-Evans. School of Biosciences, University of Nottingham, Sutton Bonnington, Leics LE12 5RD.

Introduction: Oxidative stress is a major cause of tissue degradation and thought to be a primary factor involved in the ageing process. Antioxidant defences neutralise the harmful effects of tissue oxidation. Glutathione (GSH) is a potent ubiquitous antioxidant that scavenges free radicals via the action of glutathione peroxidase. The resultant oxidised glutathione (GSSG) in then reduced by the action of glutathione reductase. The majority of circulating GSH is produced in the liver, with the kidney extracting the largest amount from the circulation. Circulating GSH concentration is elevated when organs are subjected to oxidative stress, resulting in a migration of hepatic GSH to target organs. The ageing process leads to a decrease in glutathione peroxidase activity of between 27-53% the kidney of 36 month-old mice compared to 10 months, indicating a major reduction in glutathione metabolism. An increase in oxidative stress is known to induce apoptosis. In the kidney, this can result in fewer nephrons and the development of hypertension as filtration rate is reduced. A maternal low protein diet (MLP) may exert an influence on the number of nephrons in the kidneys of offspring. Previous research has reported a reduction in nephrons in the kidneys of offspring whose mothers were fed a MLP diet. **Methods:** In this study we investigated the effects of a MLP diet (9% casein) on nephron number, glutathione redox state and markers of oxidative damage. A low protein diet was administered to maternal rats either throughout pregnancy (3 weeks gestation) or during the first (LP Early), second (LP Mid) or third (LP Late) week only. The control diet contained 18% casein. **Results:** Nephron numbers were significantly lower at 9 months compared to 4 weeks in all maternal diet groups, with the exception of LP All. Nephron numbers in LP Mid were significantly lower at 9 months compared to control ($p=0.02$). Renal GSH was significantly reduced at 9 months compared to 4 weeks, and at 18 months compared to 9 months ($p<0.05$). The ratio of GSSG to GSH was calculated to determine the relative oxidation of GSH in its function as an antioxidant. GSSG to GSH ratio at 18 months was significantly lower in both LP Mid and LP Late ($P<0.05$). Liver GSH was significantly lower in LP Mid compared to LP All ($p=0.002$) at 18 months.

	Control	LP All	LP Early	LP Mid	LP Late
Renal GSH	1.025 (0.148)	1.352 (0.159)	1.441 (0.128)†	1.266 (0.125)	1.407 (0.115)
Renal GSSG	0.618 (0.065)	0.565 (0.060)	0.636 (0.056)	0.453 (0.084)	0.499 (0.039)
Ratio (GSSG to GSH)	0.632	0.472	0.446	0.372*	0.367*

Data are mean ± S.E.M. * indicates significantly lower compared to control ($p<0.05$). † indicates significantly higher compared to control ($p<0.05$). Data generated using 2-way ANOVA. **Conclusions:** This study agrees with previous research in that nephron numbers were higher in the control group at 4 weeks compared to all MLP groups with the exception of LP Mid. This is consistent with the hypothesis that maternal undernutrition has a deleterious effect on nephron production. The decline of renal GSH in all diet groups suggests an increase in oxidative tissue damage and the increased possibility of free radical-mediated apoptosis. This could result in a reduction in nephron numbers which, when combined with the lower nephron count at birth, would severely compromise renal function and result in hypertension at maturity.

P3-058

Multi-Vitamin Supplementation during Pregnancy Changes Body Weight, Adiposity and Glucose Regulation in the Female Offspring Ignatius M.Y. Szeto, Paul J. Das, G. Harvey Anderson, Department of Nutritional Sciences, University of Toronto, 150 College Street, Toronto, Ontario, M5S 3E2, Canada.

Introduction: Over the past three decades, the public maternal consumption of vitamins through supplementation and fortification of foods have increased significantly. Also, high intake of vitamins during pregnancy can lead to epigenetic modification of gene expression in the offspring. Therefore, we hypothesized that maternal vitamin supplementation alters body composition and metabolic regulation in the offspring. **Methods:** Female Wistar rats were fed either the AIN-93G control powder diet (C) or the diet with 10X the multi-vitamin content (HV) from Day 3 of pregnancy to term. At birth, each litter was culled to 10 pups. During lactation, all mothers received the C diet. At weaning, female offspring (N = 10 / mother group) were randomly chosen to be sacrificed at weaning for fat pad mass (FPM, epididymal + peri-renal) collection. Female pups (N = 10 / mother group) were weaned to the obesity-inducing palatable liquid diet for 12 weeks. A glucose load (5g / kg body weight) was given by gavage and blood glucose was measured at baseline, and 15, 30 and 60 minutes after gavage at 0, 3, 6, and 9 weeks post-weaning. Body weight (BW) was measured weekly from birth to 12 weeks post-weaning. FPM was collected at 12 weeks in the female offspring upon sacrifice. **Results:** Female pups from the HV mothers had no BW difference at birth, but 4% lower BW at weaning (64.1 ± 0.8 g vs. 66.5 ± 1.0 g, $p = 0.07$) compared to those from the C mothers. Female pups from the HV mothers had 6% lower FPM at weaning (0.87 ± 0.03 g vs. 0.93 ± 0.02 g, $p = 0.08$). Also, they had 13% higher fasting blood glucose (8.0 ± 0.4 mmol/L vs. 7.1 ± 0.3 mmol/L, $p = 0.08$) and 13% higher blood glucose at 30 minutes (13.5 ± 0.4 mmol/L vs. 11.9 ± 0.4 mmol/L, $p = 0.01$) than pups from the C mothers. At 12 weeks post-weaning, female pups from the HV mothers had 20% lower FPM than those from the C mothers (32.7 ± 1.9 g vs. 40.9 ± 2.7 g, $p = 0.03$). BW and blood glucose response were not different after the weaning period. **Conclusion:** These data support the hypothesis that multi-vitamin supplementation during pregnancy alters growth, adiposity and glucose regulation in the female offspring, especially at the early stage of life. Also, this research suggests that maternal vitamin supplementation may predispose the offspring to an altered long-term health. This research was funded by NSERC and BMS/MJ Freedom to Discover Grant.

P3-059

Effect of Diet Composition on Pregnancy Outcome in Rapidly Growing Adolescent Sheep Raymond Aitken[1], John Milne[1], Dale Redmer[1,2] and Jacqueline Wallace[1]; Rowett Research Institute[1], Aberdeen, AB21 9SB, UK and North Dakota State University[2], Fargo, ND 58105-5727, USA

Background: In the human up to 50% of adolescents continue to grow while pregnant and in spite of larger pregnancy weight gains and increased fat stores, deliver smaller babies than non-growing adolescent mothers. Similarly, when pregnant adolescent sheep are overnourished to promote rapid maternal growth during pregnancy, growth of the placenta is impaired and results in the premature delivery of low birth weight lambs relative to moderately- fed (control) adolescents of equivalent gynaecological age. To date these effects have been achieved by feeding two levels of the same complete diet. As high protein intakes have variously been associated with the delivery of low birth weight babies, the present study evaluated the role of protein in pregnancy outcome in our adolescent sheep paradigm. **Methods**: Adolescent ewes were implanted with single embryos derived from a single sire on day 4 post-estrus. Thereafter ewes were offered an isocaloric diet (11.4 MJ ME/kg DM) containing either 12 (basic, B) or 17 (extra, E) % crude protein (CP) *ad libitum*. At day 75 of gestation, half the pregnant ewes on each protein level were switched to yield 4 groups, BB, EE, BE and EB protein. A further optimally nourished control group (C) received a moderate quantity of a ration (14% CP) designed to provide 100% of the estimated energy and protein requirement of the adolescent sheep according to stage of pregnancy. Pregnancy outcome was determined following spontaneous delivery at term. **Results**: Weekly dry matter intakes were independent of protein level in the 4 groups of *ad libitum* fed ewes and were significantly higher ($P<0.001$) than in the C group throughout.

Maternal plasma urea concentrations (determined at 14 day intervals) reflected the current CP content of the diet offered and were elevated in the 17 compared with 12% protein groups (P<0.001). Within *ad libitum* fed groups maternal plasma glucose, non-esterified fatty acid and homocysteine (Hcy) concentrations were independent of protein level. During the first two thirds of gestation, Hcy concentrations were higher (P<0.001) in moderate-intake compared with *ad libitum* fed dams, but irrespective of treatment Hcy concentrations were unrelated to birth weight. Gestation length, placental weight, lamb birth weight and initial colostrum yield were reduced (P<0.05 or less) in all *ad libitum* fed groups relative to the optimally nourished control group (Table). However within *ad libitum* groups pregnancy outcome was largely unaffected by level or timing of exposure to high protein intakes.

Table: Pregnancy outcome in relation to diet composition and maternal intake

Diet Maternal Intake	BB (n=11) *Ad-libitum*	FE (n=10) *Ad-libitum*	BE (n=7) *Ad-libitum*	EB (n=11) *Ad-libitum*	Control (n=11) Moderate
Gestation length -days	141.8±0.44^a	140.0±0.63^b	141.2±0.70^a	141.4±0.83^a	144.8±0.55^b
Lamb birth weight -g	4420±288^a	3920±300^a	4250±440^a	4230±394^a	5450±150^b
Fetal placental weight -g	367±27.8^a	256±23.5^{bc}	353±47.9^{ab}	386±47.4^a	499±28.5^b
Total fetal cotyledon wt.-g	87±6.8^a	69±8.5^a	84±10.4^a	93±11.7^a	134±10.0^b
Colostrum yield -g	174±38.3^a	152±22.3^a	182±69.1^a	254±36.6^a	693±62^b

Values are mean ± sem, within rows means with differing superscripts differ at P<0.05 or less **Conclusion**: These data imply that it is high energy intakes that are the primary cause of impaired placental development and adverse pregnancy outcome in rapidly growing adolescent sheep. *Funded by the Scottish Executive Environment and Rural Affairs Department*

P3-060

Low Protein Diet Feeding to Rats During Pregnancy Causes Apoptosis in Placenta and Reduces Feto-placental Weights Luckey C. Reed, Pandu R. Gangula, Chandrasekhar Thota and Chandrasekhar Yallampalli; Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX 77555 USA.

We have previously reported that offspring exposed to low protein diet (6%) *in utero* throughout gestation in rats resulted in the development of hypertension at around 2 to 4 months of age in both males and females. The mechanisms of this programming in rats upon exposure to low protein diet are not well characterized. In this study, we focused on the effects of exposure of pregnant rats to low protein diet (LPD) throughout gestation on placental and fetal weights and induction of apoptosis in placenta. Pregnant rats were fed with either 20% protein (control) or 6% protein (LPD) in their diet from day 1 to day 22 of gestation. Groups of 5 animals were sacrificed on day 15 or 18 of gestation and weights of fetus and placentas were recorded. Placentas were analyzed for changes in apoptotic proteins by Western blotting and data are normalized to ß-actin levels. Results showed that both placental and fetal weights were significantly lower in LPD group compared to control both on pregnancy day 15 and 18. In addition, Western blotting analysis showed that the cleaved caspase 3 protein levels were significantly higher in placentas of LPD group compared to control. Similarly, caspase 9 protein levels were also higher in these placentas. We conclude that LPD during pregnancy in rats caused programmed cell death in placentas and therefore, decreased fetal and placental weights. Further, these apoptotic changes in placenta may play a role in the programming of hypertension in the adult offspring.

Maternal Nutrition - Human

P3-061

Association between Mothers' Reported Diet and Newborn Anthropometry; Results from the French EDEN Study Marie Aline Charles, Anne Forhan, Blandine de Lauzon-Guillain, Monique Kaminski, Olivier Thiebaugeorges, Michel Schweitzer, Valérie Goua, Guillaume Magnin, Pierre Ducimetière INSERM, Institut Fédératif de Recherché 69, Paris XI University, Villejuif;. Regional Maternity, University Hospital, Nancy; Gynecology and Obstetric Department, University Hospital, Poitiers; France

Little is known in developed countries about the relationship between the mother's usual diet and her newborn's anthropometry although it is one of the proposed pathway for the early nutritional programming of later health. We therefore performed an exploratory analysis of the question using data of the ongoing EDEN study, a French study of the pre and early postnatal determinants of the child's development and health. **Subjects and methods**: Pregnant women consulting before 24 weeks of amenorrhea (WA) in the university hospitals of Nancy and Poitiers are proposed to participate into the study. Of the first 784 women included, 612 answered two food frequency questionnaires 1) on their usual diet during the year prior to pregnancy (at recruitment: 15 WA on average), and 2) during the last trimester of pregnancy (after delivery). In addition to birthweight and length, recorded newborn anthropometric data include head, arm and wrist circumferences and subscapular and tricipital skinfolds measured in triplicate by research investigators and averaged. A principal component analyses (PCA) was first performed on the mother's dietary data (average frequency of consumption of 33 food groups) to identify the main sources of variation. In a second step, mean newborn anthropometric variables were compared by quintiles of the mother's diet main axis by analysis of covariance adjusted on center, mother's age, prepregnancy body mass index, height, parity and average number of cigarettes smoked during pregnancy. The analysis was conducted on the 526 mother-newborn couples with all data available. **Results**: For the mother's diet, both before and at the end of pregnancy, the first axis of the PCA was an "energy axis" which bored positive contributions from all the food groups. The second axis opposed diet rich in fruits, vegetables and low fat food to those rich in sweetened beverages, fried potatoes, cakes and ready to eat meals (healthy diet axis). The mother's energy axis was significantly associated with the newborn sum of skinfolds both before pregnancy (from the lower to the upper quintile of the mother's diet scores: 8.6, 8.7, 8.8, 9.3, 8.9 mm p<0,02) and at the end of pregnancy (p<0,04), but there was no significant association with any of the other newborn variables. There was a strong association between the mother's healthy diet score and the newborn's head circumference before pregnancy (p<0,003) (and not at the end of pregnancy) showing a J-shape curve: from the lower to the upper quintile of the mother's scores: 34.6, 34.3, 34.2, 34.4, 34.8 cm; the corresponding percentages of newborn with head circumference = 75th percentile were: 22, 20, 24, 20, 39 %. The newborn sum of skinfolds showed an association in the opposite direction: 8.6, 9.1, 9.1, 8.9, 8.6 mm, p<0,07 corresponding to percentages of skinfolds = 75th percentile of 20, 29, 33, 29, 16 %. Birthweight and length showed no significant association with the mother's healthy diet score. The adjustment on the mother's pre-pregnancy BMI, which positive association with the healthy diet axis may disclose a reporting bias, had no influence on the results. **Conclusion**: This exploratory analysis found an association between the main sources of variation in reported diet by women in developed countries and newborn anthropometry which needs to be further investigated. A positive association between birth size and the mother's fruits and vegetables consumption has been reported before in a rural community from India.

P3-062

Comparative Study of Birth Weight of a Cohort of Diabetic Pregnant Women with that of the Normal Pregnant Women Dr. Shireen Begum, MBBS, DPH, PhD

Introduction: The diabetic pregnancy is beset with many problems but the pregnancy outcome is largely unknown in Bangladesh. The study was conducted at Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, a specialized tertiary care hospital for the diabetic pregnant women and their infants. The objective was to compare the nutritional status, pregnancy outcome and perinatal outcomes of diabetic pregnant mother and their singleton newborns and their non-diabetic counterpart. **Methodology**: This prospective study was conducted on a cohort of two hundred pre-gestational diabetic pregnant (DM) women and their matched control of non-diabetic pregnant women. The total period was spanned over a period of 19 month 2003. Pregnant women in their first trimester (<12th wk) were screened and selected for the study. The group was matched for gestational age, parity and occupation of husband. They were followed up at each trimester of the pregnancy, during labor, postpartum period and the newborn were followed up prospectively by home visits till perinatal period. **Result**: The group was not different on matching characteristics. They were significantly different in monthly income and education with lower value for DM mothers. The DM mothers were more obese (31.5% vs 18.0%, p<0.001) than the non-diabetic mother who were under weight during early pregnancy. In all three trimester the rate of anemia was found more among the DM mother and the mean hemoglobin concentration was lower in DM mothers (p<0.001) Maternal morbidity was more in DM mother in all three trimesters (p<0.001). Total weight gain was significantly higher in DM mothers (8.6 ±3.2 vs 7.9±2.5 kg) than that of the non-diabetic group. Live birth rate in the DM group found 98.0% which was 100.0% in the NDM group. Breech presentation was more in DM and cesarean section was also more in DM group. A greater proportion of infants of DM mothers were large for gestational age (LGA). Overall rate of preterm was more in DM group (41.5% vs 6.2%) and the baby born < 34 wk of gestational age was 16.2% in DM group and none in NDM group. Mean birth weight was found significantly greater (p<0.001) in the infants of diabetic mothers (IDMs) 2850.6±577.1g) than that of the NDM mothers (2651.1±344.4 g). All macrosomy baby were found among the DM and IDMs suffer more (p<0.001) from perinatal morbidity. On multiple regression analysis, newborns of diabetic mothers found to be higher weight, head, and chest and mid arm circumferences after controlling possible confounders. After controlling all the confounders, family income, and total weight gain and blood glucose level had shown significant positive effects on birth weight in both the group. **Conclusion**: Maternal glycemic status, maternal morbidity, weight gain, birth weight, gestational age and family income are the factors predicting birth weight and perinatal morbidity.

P3-063

Does Fatty Acid Intake during Pregnancy Influence Cytokine Levels in Cord Blood? Enke U, Vollhardt C, Schleussner E, Markert U, Jahreis G, Seyfarth L, Placenta-Labor, Dept. of Obstetrics, Friedrich-Schiller-University, 07740 Jena, Germany; Institute for Nutrition, Dept. of Nutrition Physiology, Friedrich-Schiller-University, 07740 Jena, Germany

Background: Recent studies showed an association between poly unsaturated fatty acids in maternal and cord blood and duration of pregnancy, fetal body length and weight, and post natal development of visual, cognitive and immune functions. Metabolites of such fatty acids are known to influence inflammatory processes, possibly including allergic reactions. Objective: The goal was to analyse a possible correlation between the recent (plasma) or the long term (erythrocyte membrane lipids) nutritional fatty acids (including the most physiologically relevant) intake,

and the level of cytokines in cord blood of term born infants. **Methods**: Cord and maternal blood (n=40) were taken after birth. Plasma and erythrocytes were isolated and stored at -86°C until lipid extraction and fatty acid methylation were performed. Therein, a total of 80 different fatty acids of carbon chain length of C10-C24, containing saturated, trans and cis mono and poly unsaturated fatty acid and conjugated linoleic acids, were analysed by gas chromatography. In fetal plasma the cytokines interferon gamma (IFN-g), tumour necrosis factor alpha (TNF-a), interleukin (IL)-10, IL-5, IL-4, IL-2 were analysed by using a human Th1/Th2 cytokine bead array (BD Biosciences). **Results**: All analysed cytokines can be detected in cord plasma. Some fatty acids influence cytokine levels in cord blood. Most effects can be found by omega (n) -3 fatty acids in membranes of red cord blood cells and maternal plasma. n-3 fatty acids like Eicosapentaenic acid, Docosapentaenic acid or Docosahexaenic acid showed positive correlation with IFN-g, TNF-a, IL-4 and IL-10 in fetal membrane lipids and with IL-5 and IL-10 in maternal plasma. In contrast, several n-6 fatty acids are negatively correlated with some Th1 cytokines and positively with Th2 cytokines. Other types, such as saturated fatty acids, trans fatty acids or conjugated linoleic acids show no or a slight negative correlation with some cytokines. **Conclusions**: Concentration of fatty acids in maternal and cord blood reflects nutrition behaviour of pregnant women. Our results suggest its influence on fetal cytokine expression and, thus, the early development of the immune system.

P3-064

Body Composition and the Insulin-like Growth Factor System in Relation to Infant Birthweight. A Study in Healthy Women During Pregnancy Elisabet Forsum, Kerstin Brismar, Moira Lewitt, Marie Löf, Hanna Olausson and Annica Sohlström; Department of Biomedicine and Surgery, University of Linköping, Linköping, Sweden, Unit for Endocrinology and Diabetes, Karolinska Institute, Stockholm, Sweden

Background: Maternal nutrition is generally considered to be important for infant birthweight but the mechanisms involved are incompletely known. Maternal body mass index before pregnancy and gestational weight gain are known as two important determinants of infant birthweight. The insulin-like growth factor (IGF) system, including IGF-I, IGF-II and six IGF-binding proteins (IGFBP 1-6) is affected by pregnancy and by nutritional factors and is considered to influence fetal growth. Thus it is of interest to study these components in serum in relation to maternal body composition and infant birthweight during human pregnancy. The components of the IGF-system are known to interact in complex ways, for example, IGFBP-3 is to some extent proteolysed during pregnancy by proteases produced by the decidua, the effect being an increased amount of free IGF-I available for receptors in, for example, the placenta a course of events that has been suggested to stimulate fetal growth. **Material and methods:** Twenty-three healthy women were studied for total body fat (TBF) before pregnancy, in gestational weeks 14 and 32 and two weeks postpartum using a two-component-model based on estimates of total body water obtained using the isotope dilution method. Infant birthweight was recorded in the delivery room. Before pregnancy and in gestational weeks 14, 32 and 35, serum levels of IGF-I and IGFBP-I were estimated using radioimmunoassay while protease activity was assessed after incubation of ^{125}I-IGFBP-3 with serum samples and subsequent electrophoresis. **Results:** TBF (%) of women before pregnancy ($r=0.48$, $p<0.05$) and in gestational week 32 ($r=0.49$, $p<0.05$) were correlated with infant birthweight. Maternal TBF (%) before pregnancy and gestational age of infants at birth explained 45% ($p<0.001$) of the variation in birthweight. When net gestational weight gain or fat retention during pregnancy was added to this model this figure was 46 and 43 %, respectively. There was a significant ($r=0.53$, $p<0.05$) correlation between TBF (kg) of women before pregnancy and their change in serum IGF-I during the first 14 weeks of pregnancy where women with the lowest body fat content before pregnancy had the largest decrease in serum IGF-I during this period of gestation. Body weight before pregnancy and serum IGF-I in gestational week 14 explained 47 % ($p<0.001$) of the variation in infant birthweight. IGFBP-I in serum was inversely correlated ($r= -0.69$ - -0.74, $p<0.001$) with TBF (%) before pregnancy and in gestational weeks 14 and 32. In gestational week 35, IGFBP-I and protease activity in serum explained 35 % ($p<0.01$) of the variation in infant birthweight. **Conclusions:** In this population of well-nourished women the maternal TBF content, rather than the amount of TBF or weight retained during pregnancy, was an important determinant of infant birthweight. The effect of the maternal TBF content on fetal growth and consequently on birthweight is apparently, at least partly, mediated by components of the IGF-system and our results indicate that a fine-tuned balance among these components provides a mechanism by which maternal nutritional status influences infant birthweight.

P3-065

Maternal Intake of Calcium Rich Foods and Circulating Micronutrient Status During Pregnancy Predict Bone Measurements of the Offspring; The Pune Maternal Nutrition Study Anjali Ganpule[1], Chittaranjan Yajnik[1], Shobha Rao[2], David Fisher[3], Aaasawari Kanade[2], Himangee Lubree[1], Vaishali Deshpande V[1], Sadanand Naik S[1], Charu Joglekar [1], Cyrus Cooper[3], Caroline Fall[3]; [1] KEM Hospital Diabetes Unit, 6th floor, Banoo Coyaji Building, KEM Hospital, Rasta Peth, Pune 411007, Maharashtra, India [2] Department of Biometry, Agharkar Research Institute, Agarkar Road, Pune 411004, Maharashtra, India [3] MRC Epidemiology Resource Centre,University of Southampton, Southampton General Hospital, Southampton SO16 6YD, UK

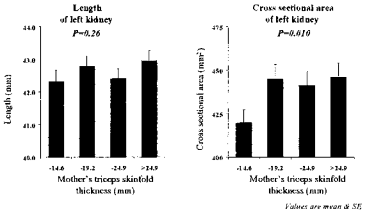
Background: Bone measurements in offspring are thought to be determined by both genetic factors and the post-natal environment. Recent evidence suggests effects of the pre-natal environment, including maternal nutrition. The Pune Maternal Nutrition Study, a population-based longitudinal study in rural Western India studied maternal nutrition during pregnancy and bone measurements of the children. **Aim:** To study the relationship between maternal size, nutrition and Bone Mineral Density (BMD) of the offspring at 6y. **Methods:** We measured maternal anthropometry, nutritional intake, physical activity and circulating nutrients in 797 pregnant women at 18 and 28 weeks gestation. Babies were measured in detail at birth and, infant feeding and weaning practices were recorded. We measured body composition and BMD (Total body, Dual Energy X-ray Absorptiometry) in 695 children (91% of survivors) and their parents, 6y after the delivery. **Results:** The children's mean height and weight were 109 cm and 16 kg. Total body BMD was 0.78 g/cm^2 in boys (n=369) and 0.77 g/cm^2 in girls (n=326). Children of mothers who consumed milk or milk products frequently (2-3 times /day) had higher BMD (0.79 g/cm^2 Vs rare consumers (<2 times/ month) 0.78 g/cm^2, $p<0.01$). Higher maternal erythrocyte folate concentrations at 28 weeks gestation and lower plasma total homocysteine concentration during pregnancy predicted higher BMD in the children ($p<0.05$ both). Lower maternal parity, higher body fat mass and lower physical activity during pregnancy predicted higher BMD of children ($p<0.01$ for all). Maternal habit of chewing tobacco was not related to the children's BMD. The child's birthweight, length and placental weight, and current age, height and weight were positively correlated with BMD ($p<0.001$ for all). Duration of breast-feeding was not related. The height and BMD of both parents and socio-economic status were positively correlated with the BMD of the children ($p<0.01$ for all). In multiple regression analyses, higher maternal frequency of intake of milk and milk products at 28 weeks (<0.001) and BMD of both parents (Regression slope: Mother 0.21 (95% CI 0.16 to 0.25), father 0.14 (95% CI 0.10 to 0.17) $p<0.001$ for all) were independent predictors of the child's BMD. Maternal consumption of milk and milk products accounted for around 2% of the total 40% variance. **Conclusion:** A sizeable proportion of the BMD of children is determined by 'environmental' factors, with a potential for modification. Higher maternal intake of calcium-rich foods during pregnancy and higher folate and lower homocysteine status predict higher BMD in the child. Intrauterine life is important in determining the bone health.

P3-066

Mother's Body Composition Influences Fetal Kidney Size and Shape in Late Gestation Kanchan Mukherjee, Mark Hanson, Sarah Crozier, Hazel Inskip, Keith Godfrey & the SWS Study Group; Centre for Developmental Origins of Health and Disease and MRC Epidemiology Resource Centre, University of Southampton, UK.

Background: Nephron number is determined during intra-uterine development and there is now evidence linking low nephron number with hyperfiltration injury and susceptibility of adult hypertension. There is also evidence that low nephron number can alter kidney size and shape, and a preliminary study found that patients with hypertension tend to have long, thin "sausage-shaped" kidneys. As maternal thinness and lower triceps skinfold thickness have been associated with hypertension in the offspring, we hypothesised that maternal thinness might be associated with low nephron number and impaired renal growth. To investigate this hypothesis, we have examined the influence of maternal body composition on fetal kidney size and shape. **Methods**: In the Southampton Women's Survey, the mother's height, weight and skinfold thicknesses have been measured before pregnancy. At a mean (10th-90th centiles) gestation of 241 (236-244) days, we measured fetal kidney length, width and antero-posterior diameters; cross sectional area was derived from width and antero-posterior diameters. Within this narrow gestation period, the duration of gestation was not associated with kidney size. We achieved measurements of the right kidney in 520 fetuses and of the left kidney in 529. **Results**: Length and cross sectional area of both fetal kidneys were larger in boys than girls ($P<0.005$). Taller mothers tended to have fetuses with greater right and left kidney length (both $P=0.004$) but mother's height was not associated with the cross sectional area of either kidney. Thinner mothers with lower triceps skinfold thickness before pregnancy tended to have fetuses with smaller cross sectional area of both right and left kidneys (both $P=0.01$), but maternal triceps skinfold thickness was not associated with kidney length; Figure 1 shows data for the left kidney.

Figure 1 Left kidney length and cross sectional area at 34 weeks gestation in relation to maternal triceps skinfold thickness before pregnancy (n=523 SWS pregnancies)



Conclusion: Thinner mothers with lower triceps skinfold thickness tend to have fetuses with "sausage-shaped" kidneys that are relatively narrow for their length. This may reflect lower nephron numbers and supports the hypothesis that impaired

renal development may in part underlie the link between maternal thinness and raised offspring blood pressure.

P3-067

Maternal Body Composition has Differing Associations with Fetal Liver Volume and a Measure of Neonatal Body Composition. Guttorm Haugen,[1] Sarah Crozier,[2] Mark Hanson,[1] Hazel Inskip,[2] Torvid Kiserud,[3] Keith Godfrey[1,2] & the Southampton Women's Survey Study Group. [1]Centre for Developmental Origins of Health and Disease & [2]MRC Epidemiology Resource Centre, University of Southampton, Southampton, UK; and [3]Institute of Clinical Medicine, Department of Obstetrics & Gynecology, University of Bergen, Norway

Objectives Inferences about fetal liver growth are often made from measurement of fetal abdominal circumference, ignoring the contributions of subcutaneous fat deposition. To investigate if the mother's body composition has differing effects on fetal liver growth and adiposity, we have examined the associations between the mother's height and measures of her fat and lean mass before pregnancy and the late gestation liver volume and neonatal mid-upper arm circumference and triceps skinfold thicknesses of her infant. **Methods** We studied 39 singleton uncomplicated pregnancies taking part in the Southampton Women's Survey, a population-based study of nutrition before and during pregnancy. The mother's height, weight, mid-upper arm circumference and four-site skinfold thicknesses had been measured before pregnancy. At 36 weeks gestation, we measured fetal liver volume (3D ultrasound) and abdominal circumference. After delivery, we measured the mid-upper arm circumference and triceps skinfold thickness of the neonate. **Results** Fetal abdominal circumference was highly correlated with fetal liver volume ($r=0.79$, $p<0.0001$) and neonatal mid-upper arm circumference ($r=0.69$, $p<0.0001$) and triceps skinfold thickness ($r=0.59$, $p=0.0001$). In a simultaneous analysis, liver volume and neonatal triceps skinfold thickness had independent associations with fetal abdominal circumference. Liver volume was strongly associated with neonatal mid-upper arm circumference ($r=0.59$, $p=0.0001$), but less strongly with neonatal triceps skinfold thickness ($r=0.41$, $p=0.01$). Taller mothers' height was associated with greater fetal liver volume ($r=0.37$, $p=0.02$) and neonatal mid-upper arm circumference ($r=0.33$, $p=0.04$), but not associated with neonatal triceps skinfold thickness ($r=0.07$, $p=0.66$). Within this sample, measures of maternal fat mass (triceps and sum of skinfold thicknesses) were not associated with fetal liver volume and neonatal mid-upper arm circumference, or with neonatal triceps skinfold thickness. Lower maternal arm muscle area, mid-upper arm circumference and body mass index were associated with lower neonatal triceps skinfold thickness ($r=0.48$, $p=0.002$; $r=0.34$, $p=0.04$ and $r=0.35$, $p=0.03$, respectively), but these measures of maternal lean mass had no significant associations with fetal liver volume or neonatal mid-upper arm circumference. **Conclusions** Fetal liver volume and adiposity both contribute to measured fetal abdominal circumference. However, particular aspects of the mother's body composition have differing associations with the liver volume and adiposity of the fetus. In our study, taller mothers had infants with greater liver volume and mid-upper arm circumference, but the mother's own lean mass was not related to either of these measures of the infant's lean mass. Mothers with a lower lean mass did, however, have infants with thinner triceps skinfold thickness. Our data suggest that detailed measurements of fetal organ size and body composition are required to understand the influence of the mother's body composition on the growth and development of her infant.

P3-068

Association of Antioxidative Vitamin and Oxidative Stress Levels in Pregnancy with Infant Growth until the Second Year of Life

Juhee Hong, Eun Ae Park, Young-Ju Kim, Hwa Young Lee, Bo-Hyun Park, Eun-Hee Ha, Kyoung Ae Kong, Hyesook Park; Department of Preventive Medicine, Department of Pediatrics, Department of Obstetrics & Gynecology, Department of Anatomy, College of Medicine, Medical Research Center, Ewha Womans University, Seoul, Korea

Backgrounds: Insufficient maternal nutritional status during the critical stage of fetal development subsequently contributes to growth retardation in infants and adverse health outcomes in adult life. Whereas there are numerous literature relating the impacts of maternal nutritional status on subsequent birth outcome, much less is known about the long-term impacts on infant growth after birth. Therefore, we aims to construct Infant Growth Cohort for evaluating whether oxidative stress and antioxidant vitamin levels in midterm pregnancy have an adverse effect on postnatal growth by the 24 months. **Methods and materials**: We constructed Ewha Pregnant Women Cohort to investigate pregnant women who visited hospital for prenatal care during gestational weeks 24-28. After delivery, we enrolled their healthy infants and constructed prospective Ewha Infant Growth Cohort from September 2001 to April 2004. We excluded mother-and-child pairs in which the mother had experienced hypertension or diabetes during pregnancy and had multiple births for this study, which gave us 291 mother-and-child pairs for analysis. For exposure variables, we measured the levels of maternal serum antioxidant, such as vitamin A, C, E, and folate and also the levels of maternal urinary oxidative stress, 8-hydroxyguanosine (8-OHdG) and malondialdehyde (MDA) at 24~28 weeks of pregnancy. For outcome variables, we measured infant weights and heights at birth and at 6, 12, 18, and 24 months postnatally. We obtained birth outcomes by review of medical chart and postnatal outcomes by using medical charts and mailing questionnaires. We applied repeated-measures ANOVA with PROC MIXED to assess the significance of differences. **Results**: For the weights of infants, we followed 570, 225, 171, 78, and 54 infants at 0, 6, 12, 18 and 24 months respectively. The mean body weights were 3256.6 g at birth, 8372.9 g at 6 months, 10057.4 g at 12 months, 11378.9 g at 18 months, and 12575.9 g at 24 months. For the heights of infants, there were 561, 153, 151, 66, and 50 infants, and mean heights were 49.3 cm at birth, 69.1 cm at 6 months, 76.7 cm at 12 months, 81.3 cm at 18 months, and 87.6 cm at 24 months. For antioxidative vitamin, the mean infant weights of 0, 6, 12, 18, and 24 months were lower in mothers with lower folate levels and there was a statistical significance ($p=0.02$). When adjusting for infant sex and gestational age, there were also decreased weights with lower folate levels, but marginal significance was shown ($p=0.05$). Retinol, vitamin C, and tocopherol also indicated relatively positive association, but statistical significances were not found. The mean heights showed same trends to weights, excepting that statistical significance was not found in folate levels. In terms of oxidative stress, weights and heights at birth and at 6~24 months in higher groups of MDA levels were significantly lower with the adjustment of infant sex and gestational age ($p=0.04$). Similarly, high levels of 8-OhdG showed reduced weights at birth and after birth, and statistical significance was shown only in heights ($p=0.04$). **Conclusions**: During pregnancy, the importance of preventing folate deficiency was explicitly shown. The fact that excess maternal oxidative stress, MDA or 8-OhdG, caused consistent declines of infant weights or heights until the second year of life, necessitates the preventive strategy to diminish oxidative stress of pregnant women. However, there were high rate of follow up loss and various measurement errors. Therefore, we need to make efforts for compete follow up and valid and reliable measurement.

P3-069

Pre-Pregnant Maternal Dieting Status and Its Relation to Gestation Length and Placental COX-2 Protein Levels JF Johnstone[1], RM Lewis[5], S Crozier[4], M Zelsman[1], H Inskip[4], M Hanson[5], JRG Challis[123], KM Godfrey[4,5] and the Southampton Women's Survey Study Group. Departments of Physiology[1], Obstetrics and Gynecology[2] and Medicine[3], University of Toronto, Ontario; MRC Epidemiology Resource Centre[4] and Centre for Developmental Origins of Health and Disease[5], University of Southampton, UK.

Objective: In sheep, nutritional restriction to produce a 15% reduction in maternal weight before conception leads to increased pre-term delivery and a precocious activation of the fetal hypothalamic-pituitary-adrenal axis. However, a milder nutritional restriction around conception is associated with increased duration of gestation; the underlying mechanisms are unclear, but an interaction between glucocorticoids (GC's) and prostaglandins (PG's) may be involved as this has a critical role in the timing of birth. The level of bioactive GC's in fetal tissues is controlled in part through metabolism by the 11β hydroxysteroid dehydrogenase (11β HSD) isoenzymes. Previously, we found that the expression of placental 11β HSD-2 was decreased in women who reported dieting to lose weight before pregnancy, suggesting that the fetuses in these pregnancies may have been exposed to inappropriate levels of maternal cortisol. In late gestation, cortisol is known to upregulate COX-2, which is responsible for PG synthesis. In this study, we examined the hypothesis that the duration of gestation will be increased in women who reported dieting to lose weight prior to pregnancy, and that changes in placental cortisol metabolism in such women lead to alterations in placental COX-2 protein expression. **Methods:** Within the population-based Southampton Women's Survey (SWS), we related maternal dieting behaviour to gestation length in a sample of 392 women whose estimated date of conception was set from menstrual data confirmed by an early ultrasound scan and who had a spontaneous onset of labour and delivered after 259 days or more gestation (37 weeks). In the SWS, the women have been characterised before pregnancy and asked whether they were currently trying to lose weight by dieting. Snap frozen placental, amnion and chorion samples were taken from a subset of 50 uncomplicated term SWS pregnancies to determine the effect of dieting behaviour on COX-2 and 11β HSD-2 protein expression, measured using western blot analysis. **Results:** Within the 392 term deliveries, mean (95% confidence interval) duration of gestation averaged 2.8 (0.9 - 4.8) days longer in the 87 women who reported dieting to lose weight before pregnancy ($p=0.004$). Within the subset of 50 pregnancies, placental COX-2 protein levels were higher (ROD 3.52+/-0.34 vs. 2.23+/-0.16, $p=0.006$) in the 8 women who reported that they were trying to lose weight by dieting prior to pregnancy; however, there were no associations between COX-2 in the fetal membranes and maternal dieting behaviour. In placental tissue, COX-2 protein levels were negatively correlated with 11β HSD-2 protein expression ($r=-0.37$, $p=0.008$), and positively correlated with gestational length ($r=0.38$, $p=0.048$). **Conclusions:** The fetal capacity to produce PG's at the end of gestation is elevated in women who reported dieting before pregnancy due to an increase in COX-2 protein expression. This upregulation could be controlled by a decrease in placental cortisol metabolism by11β HSD-2 in these pregnancies. Paradoxically, the duration of gestation is increased in these pregnancies, and we speculate that this could reflect a block in the parturition cascade at the end of pregnancy contributing to an overactivity of the feedforward loop that produces PG's.

P3-070

Lipid Peroxidation and Antioxidant Status in Uncomplicated and Preeclamptic Pregnancies Md. Zakir H. Howlader, Tanzir A. khan and Yearul Kabir; Department of Biochemistry and Molecular Biology, University of Dhaka, Bangladesh, Department of Family Sciences, College of Women, Kuwait University, Kuwait.

Background: Preeclampsia is a pregnancy-specific disorder that affects approximately 6-8% of all gestations and is the leading cause of fetal growth retardation, infant morbidity and mortality, premature birth and maternal death. Although preeclampsia was first described over 100 years ago, little is known about the pathophysiology of this disease. Several theories, which are not mutually exclusive, attempt to explain the pathophysiology of preeclampsia. Increasing evidence also supports the hypothesis that excessive production of oxygen and nitrogen-based free radicals ("oxidative stress") are involved in the pathophysiology of preeclampsia. Eclampsia is the third major cause of maternal death in Bangladesh (16%) proceeded by hemorrhage and sepsis. The objective of the study is to assess lipid peroxidation and antioxidant status in uncomplicated and complicated pregnancy (preeclampsia). **Methods**: Lipid profile, thiobarbituric acid reactive substances (TBARS), lipid hydroperoxide, total antioxidant status, vitamine C of twenty five healthy nonpregnant women, twenty two healthy women with uncomplicated pregnancy, and twenty five pregnant women with preeclampsia were measured and compared. Plasma total cholesterol, HDL-cholesterol and TG, were determined by the commercially available kits. Serum ascorbic acid was measured by dinitro phenyl hydrazine method with modification. TBARS value was determined according to the method of Yagi. Lipid hydroperoxide value was determined by colorimetric method based on the oxidation of ferrous to ferric ion in the presence of xylenol orange. Total antioxidant status was determined by using commercial kit. **Results**: It was observed that TBARS and lipid hydroperoxide were significantly ($P<0.001$) increased and total antioxidant status (TAS) and vitamin C level were significantly ($P<0.001$) decreased in preeclampsia group compared to uncomplicated pregnancy but no such changes were observed in uncomplicated pregnancy compared to the nonpregnant control. Cholesterol TG, LDL level were elevated and HDL level was normal in uncomplicated pregnancy group compared to nonpregnant control, but in preeclampsia group HDL were lowered and TG were elevated compared to uncomplicated pregnancy that correlate with lipid peroxidation rate. **Conclusions**: The results of these investigation suggested that oxidants, antioxidants are altered in preeclampsia and that may play a significant role in the pathophysiology of preeclampsia.

P3-071

Intake of Vitamin C in Pregnancy and Risk of Preeclampsia: Prospective Study Among 45,063 Women Åse K Klemmensen[1,2], Ann Tabor[3], Marie Louise Østerdal[1], Vibeke Kildegaard Knudsen[1], Thorhallur Ingi Halldorsson1, Tina Broby Mikkelsen[1] and Sjurdur Frodi Olsen[1]. From the Department of [1]Obstetrics and Gynecology, H:S Hvidovre Hospital, University of Copenhagen, [2]the Maternal Nutrition Group, Danish Epidemiology Science Centre, Statens Serum Institut and [3]the Ultrasound Clinic, Juliane Marie Center, H:S Rigshospitalet

Background: We examined the association between the risk of preeclampsia and the mid-pregnancy intake of vitamin C and vitamin E. Among the 101,039 participants in the Danish National Birth Cohort 45,063 (45%) had during pregnancy contributed complete information on intake of food, food-supplements and factors considered as confounders. **Methods:** Information about preeclampsia and hypertension during pregnancy collected via interviews post partum was combined with preeclampsia diagnoses reported to the Danish National Patient Registry. **Results:** We observed 795 (1.8%) cases with preeclampsia and 182 (0.4%) cases with severe preeclampsia. 89% reported taking multivitamin tablets. Estimated aggregated (diet + supplements) mean intakes of vitamin C were 219 (SD 149) mg per day and for vitamin E 17 (SD 20) mg per day. An intake below the Nordic Recommended Dietary Allowance was observed in 2.8% (1,278) for vitamin C and in 10.5% (4,716) for vitamin E. We detected an increased risk of preeclampsia, among women with a vitamin C intake below 70 mg/day. Adjusted odds ratio (95% CI) was 0.61 (0.38,0.99) for intake in the range 210 – 275 mg/day compared with < 70 mg/day. The association was stronger as regards severe preeclampsia. For vitamin E, there was no association with the risk of preeclampsia. **Conclusions:** In conclusion we identified consumption of vitamin C below the Nordic Recommended Dietary Allowance as a risk factor for preeclampsia, observing a Western population. It would seem reasonable, based on these data, to emphasize that women should comply with the current dietary recommendations regarding vitamin C.

P3-072

Intake of Iron in Pregnancy and Fetal Growth Vibeke K. Knudsen, Tina B. Mikkelsen, Sjurdur F. Olsen; Maternal Nutrition Group; Danish Epidemiology Science Center; Statens Serum Institut; Denmark

Background Conflicting results exist on whether iron intake in pregnancy may have a protective effect against preterm birth and low birth weight. In Denmark, all pregnant women are advised to take supplementary iron (50-70mg per day) from week 20 of gestation. We examined the association between dietary iron and iron supplements and low birth weight and preterm delivery in a large prospective study. **Methods** In the Danish National Birth Cohort, detailed information on intake of iron supplements was collected in week 25 of gestation. Further, a large number of gestational, lifestyle and socio-demographic variables was collected. Dietary intake and use of dietary supplements was assessed in week 25 of gestation by means of a semi-quantitative food frequency questionnaire. Intake of iron from the diet was adjusted for energy according to the residual method, and the women were divided into quintiles according to iron intake. Intake of iron from supplements was divided into following groups: 0 mg, 1-29 mg, 30-59 mg 60-89 mg, 90-119 mg and 120 or more mg per dagy. Information about gestational age and birth weight was registered in the National Patient Register. Low birth weight (LBW) was defined as birth weight less than 2500g, and preterm delivery was defined as delivery before 259 days of gestation. Confounder adjusted analyses of risk of preterm delivery and LBW were carried out by means of multiple logistic regression analyses (SAS Institute Inc., Cary, USA) **Results** In the univariate analyses (adjusted only for gestational length) women in the lowest quintile iron intake had an increased risk of preterm LBW (OR 1.46, 95%C.I.: 1.04-2.03); the association was weakened in the confounder adjusted analyses. Mean birth weight and gestational length increased with increasing iron intake; the largest difference was seen between the lowest and highest quintile (40g, 95% C.I.:25-56g). Women who did not take iron supplements had significantly lower birth weight than the women with the highest intake (27g, 95% C.I.: 5-48 g), but no association were seen between iron from supplements and preterm LBW. **Conclusions** We found a possible protective effect of iron from diet on LBW, but no effect of iron from dietary supplements. As the main part of a woman's iron supply comes from supplements, these results indicate that it there is no effect of iron supplementation in late pregnancy. If this is the case, the effects on birth weight reported in other studies may be due to supplementation in early pregnancy. Another possible explanation is that the iron from the diet is a proxy of a dietary factor which may protect against low birth weight The study was supported by the March of Dimes Birth Defects Foundation and EU (NUTRIX QLK 1CT 2000-83, and Earnest contract number 7036)

P3-073

The Association Between Birthweight and Puberty Onset Among Girls in the United States Susan Olivo Marston[1], Barry Graubard[2], and Michele Forman[3] [1]Division of Cancer Prevention, [2]Division of Cancer Epidemiology and Genetics, [3]Center for Cancer Research, The National Cancer Institute, The National Institutes of Health, Bethesda, MD 20892

High birthweight has been associated with an increased breast cancer risk. Additionally, research has established a link between puberty, specifically age at menarche, and breast cancer risk although the mechanisms underlying these associations are not known. It is possible that puberty lies in the causal pathway from birthweight to breast cancer risk. In other words, perhaps high birthweight babies undergo puberty onset earlier correlating with a younger age at menarche, thus leading to an increased breast cancer risk. Therefore, examining possible associations between these factors are of great clinical importance, especially as the median age of puberty onset has decreased in the past several decades among girls in the United States. In the current study, we investigated the hypothesis that birthweight is associated with puberty onset among girls in the United States. The study data is from the Third National Health and Nutrition Examination Survey (NHANES), conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC) from 1988 through 1994. This study provided national estimates of health and nutrition for the U.S. population ages 2 months and older. The current study, all girls between the ages of 8 and 11 years that underwent both a home interview, and the medical examination for Tanner staging were included (n=1,077). Of these participants, 23.3% of them were 8 years old, 25.7% were 9 years old, 24.4% were 10 years old, and 26.8% were 11 years old. Additionally, 26.7% were White, 33.3% were Black, and 35.8% were Mexican-American. The main outcome of this study was puberty onset as determined by Tanner staging. This involved assessing both breast development and pubic hair growth. Puberty onset was defined as a Tanner stage above 1 for either or both categories, while prepubertal was defined as girls with a Tanner stage of 1 for both breast and pubic hair development. The association between birthweight and puberty onset was assessed using a logistic regression model that controlled for age, ethnicity, and height. As expected, an increase in age was significantly associated with puberty onset. Additionally, both Black and Mexican-American girls had increased odds ratios (ORs) for puberty onset, compared with white girls. Finally height was positively associated with puberty onset. Examination of a possible association between birthweight and puberty onset illustrated an increased OR of 1.23 for low birthweight babies, while high birthweight babies had a decreased OR (0.87) for puberty onset, although neither association was significant. Secondary analysis, however, showed an increased OR for low and high birthweight babies compared to average birthweight babies when examining only breast development, although again this finding was not statistically significant ($p=0.28$ and 0.15, respectively). In conclusion, high birthweight was not significantly associated with puberty onset among girls ages 8-11 in the NHANES III study. Limitations of the study included possible residual confounding and information bias, both which could have obscured a possible association between birthweight and puberty onset. Further research needs to be done to elucidate the mechanisms underlying the effect of birthweight on breast cancer risk and how modifications in puberty onset affect breast cancer risk.

P3-074

Maternal Diet in Early and Late Pregnancy Related to Weight Gain and Birth Outcome Anna S. Olafsdottir ,[1,3] Gudrun V. Skuladottir,[2] Inga Thorsdottir,[3] Arnar Hauksson,[4] Laufey Steingrimsdottir[1;1]Public Health Institute of Iceland, [2]Department of Physiology, Faculty of Medicine, University of Iceland, [3]Unit for Nutrition Research, Landspitali – University Hospital & Department of Food Science , University of Iceland, and [4]Center of Prenatal Care, Reykjavik Health Care Center, Iceland.

Background: Weight gain during pregnancy is one of the main factors related to birth outcome, with birthweight being highly correlated to maternal weight gain. Adequate weight gain is important for optimal perinatal outcome, but high weight gain in pregnancy has been related to complications during pregnancy and delivery and macrosomia, and low weight gain to retarded fetal growth and preterm birth. Icelandic women tend to be tall and give birth to infants at the upper end of the scale for birth size worldwide. Identification of the dietary factors along with other factors influencing gestational weight gain and birth outcome is of utmost importance for practitioners to guide their clients through an optimal pregnancy and prevent even further increase in the obesity epidemic. The relationship of changes in food consumption during pregnancy and consequent weight gain has not been studied. With this in mind the aim of our present study was to investigate the association of maternal dietary intake in early and late pregnancy and anthropometric factors with excessive or suboptimal gestational weight gain as well as pregnancy outcomes. **Methods**: The dietary intake of 495 healthy pregnant Icelandic women was estimated with a semi-quantitative food frequency questionnaire covering food intake together with lifestyle factors for the previous three months. Questionnaires were filled out at between 11 and 15 weeks and between 34 and 37 weeks gestation. Comparison of birth outcome between the three weight gain groups was made with ANOVA and Bonferroni post hoc tests. Dietary factors related to at least optimal and excessive weight gain during pregnancy were represented with logistic regression controlling for potential confounding. **Results**: Of the women, 26% gained suboptimal and 34% excessive weight during pregnancy. Women with at least optimal, compared with women with suboptimal, weight gain were more likely to increase their energy intake (OR= 3.32, CI=1.81-6.09, P<0.001) and drink more milk in late pregnancy (OR=3.10, CI=1.57-6.13, P=0.001). The same dietary factors were related to excessive, compared with optimal weight gain. Furthermore, eating more sweets early in pregnancy increased the risk of gaining excessive weight (OR=2.52, CI=1.10-5.77, P=0.029). Women with a BMI of 25.0-29.9 before pregnancy were most likely to gain excessive weight (OR=7.37,CI 4.13-13.14, P<0.001). Of the normalweight women, women gaining suboptimal weight had shorter gestation (280±8 vs. 281±8 and 284±7 days, P=0.008) than women gaining optimal and excessive weight, respectively. They also gave birth to smaller babies (birthweight 3591±447 vs. 3764±436 and 3872±471 g, respectively, P<0.001). Within the group of overweight women, there was only a significant difference between babies' birthweight of women gaining suboptimal vs. excessive weight (3594±443 vs. 3918±526 g, P=0.029). **Conclusion**: Suboptimal maternal weight gain is related to smaller infants with shorter gestational duration, whereas excessive weight gain does not add significantly to the infants' size compared with optimal weight gain. Pregnant women should receive guidance to opt for the choice of healthy foods in appropriate amounts to encourage optimal weight gain. Women who are overweight before pregnancy need special attention and support as they are most likely to gain excessive weight and therefore most likely to suffer pregnancy and delivery complications and struggle with increasing overweight and obesity after giving birth.

P3-075

Association of Milk Consumption in Pregnancy with Increased Foetal Growth Olsen SF, Halldorsson TI, Willett WC, Knudsen VK, Mikkelsen TB, Gillman MW, Olsen J and the NUTRIX Consortium; Maternal Nutrition Group, Danish Epidemiology Science Centre, Statens Serum Institut, 5 Artillerivej, DK-2300 Copenhagen S, DENMARK; Harvard School of Public Health and Harvard Medical School, Boston.

Background Some evidence suggests that maternal consumption during pregnancy of cow's milk, which contains many growth-promoting factors, may increase fetal growth. We examined the relationship between intake of cow's milk in pregnancy and foetal growth. **Material and methods** In the Danish National Birth Cohort we collected information prospectively on dietary intake in mid-pregnancy from 45,754 women using a food frequency questionnaire. Data on covariates were obtained through computer assisted telephone interviews, and birth outcomes were ascertained through linkages to national registries. Analyses were adjusted for gestational age at birth; mother's parity, age, body mass index (BMI), height, smoking and energy intake; father's height; and family socio-economic status. **Results** On average, women reported consuming 2.6 glasses of milk per day (SD 1.89) and birth weight was 3588 (SD 571) grams, birth length 51.9 (SD 5.8) cm, head circumference 34.6 (SD 5.6) cm, abdominal circumference 32.1 (SD 7.3) cm, and placental weight 643 (SD 186) grams (n=45,754). After adjustment for covariates, milk intake exhibited a positive graded relationship with birth weight, with an increment of 100 grams (95% confidence interval 62 to 137, p, trend <0.0001) comparing women consuming six or more glasses per day to those consuming no milk. Strong, graded relationships were also seen for abdominal circumference (total increment 0.52 cm (0.34 to 0.69, p, trend <0.0001)) and placental weight (24 grams (13 to 36, p, trend <0.0001)), but weaker associations were seen for birth length (total increment 0.17 cm (-0.01 to 0.35, p, trend , <0.0001)) or head circumference (0.08 grams (-0.05 to 0.20, P, trend = 0.003)). Birth weight exhibited clear relationships with daily intakes of protein and carbohydrates derived from milk, whereas no association could be detected between birth weight and daily intake of fat from milk. **Conclusion** We hypothesise that cow's milk contains water-soluble substances that, when ingested in pregnancy, increase visceral growth with less effect on linear or brain growth of the foetus. The study was supported by the March of Dimes Birth Defects Foundation and grants from EU FP5 and FP6 (NUTRIX QLK1CT2000-83, and EARNEST (contract number 7036)).

P3-076

Preeclampsia Risk in Relation to Vitamin D and Calcium Intake and to Season of the Year Østerdal ML, Klemmensen A, Tabor A, Hypponen E, Gillman MW, Knudsen V, Mikkelsen TB, Halldorsson TH, and Olsen SF; Maternal Nutrition Group, Danish Epidemiology Science Centre, Statens Serum Institut, 5 Artillerivej, DK-2300 Copenhagen S, DENMARK; Rigshospitalet, Copenhagen, DENMARK; Institute of Child Health, University College London, UK; and Harvard Medical School, Boston, USA.

Background Meta-analyses of RCTs have suggested that calcium supplementation in pregnancy is capable of preventing preeclampsia, although the effect has not been detectable in all trials (Atallah et al. Cochrane Database Syst Rev 2002). Recently it has also been proposed that vitamin D could protect against preeclampsia (Hypponen. Nutrition Reviews, In Press). **Material and methods** In the Danish National Birth Cohort we collected prospectively information on dietary intake in mid-pregnancy from 70,183 women using a food frequency questionnaire. Data on covariates were obtained through computer assisted telephone interviews, and birth outcomes were ascertained through linkages to national registries. Analyses were adjusted for mother's parity, age, body mass index (BMI), height, smoking; and family socio-economic status. Calcium intake was dichotomised at the lowest quintile (cut-off 1040 mg/d), whereas vitamin D was dichotomised at the highest quintile (cut-off 4.5 mcg/d). Women who had the whole 1st trimester of pregnancy between October and March (and who supposedly were able to produce less amounts of endogenous vitamin D due to less sun exposure) were categorised as winter pregnancies. Decisions regarding cut-off points used were in part data driven. The study was approved by the Danish Scientific-Ethical Committee system. **Results** In the aggregated data set, non-winter pregnancies were at increased risk of preeclampsia, with an OR of 1.14 (95% CI 1.01 to 1.29, n = 54,438), whereas vitamin D (OR x, 95% CI x to y) and calcium (OR x, 95% CI x to y) intake had no independent association with preeclampsia. Among winter pregnancies, and among women with low calcium intake (n =2,769), having a high intake of vitamin D was associated with reduced risk of preeclampsia (OR 0.51, 95% CI 0.24 to 1.08), whereas among women with high calcium intake, no such association was seen (OR 1.16, 95 CI% 0.86 to 1.56). Likewise, among winter pregnancies, and among women with low vitamin D intake (n =10,816), having a high intake of calcium was associated with reduced risk of preeclampsia (OR 0.67, 95% CI 0.51 to 0.88), whereas among women with high vitamin D intake, no clear association or possibly a reverse association was seen (OR 1.50, 95% CI 0.71 to 3.18). Among non-winter pregnancies (n=40,858), similar, albeit weaker associations were seen. **Conclusion** In women with limited sun exposure during the first trimester, calcium may protect against preeclampsia if the woman has a relatively low intake of vitamin D, whereas vitamin D may protect against preeclampsia if the woman has a relatively low intake of calcium.
The study was supported by the March of Dimes Birth Defects Foundation and EU (NUTRIX QLK1CT2000-83, and EARNEST contract number 7036).

P3-077

Predictors of Dietary Quality During the 1st Trimester of Pregnancy Sheryl L. Rifas-Shiman[1], Janet W. Rich-Edwards[1,2], Ken P. Kleinman[1], Emily Oken[1], Matthew W. Gillman[1,3] [1]Department of Ambulatory Care and Prevention, Harvard Medical School/Harvard Pilgrim Health Care, Departments of [2]Epidemiology and [3]Nutrition, Harvard School of Public Health; all in Boston, MA

Background: Maternal diet may influence outcomes of pregnancy and childhood, such as length of gestation, fetal growth, birth defects, preeclampsia, and offspring cognitive development, blood pressure, adiposity, and atopic disease. Data on determinants of food and nutrient intake during pregnancy are scarce. **Objective:** To examine the relationship between maternal characteristics and diet quality during the 1st trimester of pregnancy. **Methods:** As part of the ongoing US prospective cohort study Project Viva, we studied 1777 women who completed a food frequency questionnaire that assessed dietary intake during the 1st trimester of pregnancy. We used linear regression models to examine the relationship between several maternal characteristics selected *a priori*, namely age, pre-pregnancy body mass index (BMI), parity, education, and race/ethnicity with dietary intake. We used the Alternative Healthy Eating Index (AHEI), slightly modified for pregnancy, to measure diet quality on a 90-point scale with each of the following 9 components contributing 10 possible points: vegetables, fruit, ratio of white to red meat, fiber, *trans* fat, ratio of polyunsaturated to saturated fatty acids, and folate, calcium, and iron from foods. **Results:** Mean (SD, range) AHEI score was 61 (10, 33-89). After adjusting for all characteristics simultaneously, participants who were

older, leaner, nulliparous and more educated had higher AHEI scores, but black and white participants had similar AHEI scores (table).

Variable	Age			BMI (kg/m^2)			Parity			Education			Race/ethnicity	
Category	<25	25-<35	≥35	<25	25-<30	≥30	0	1	≥2	<HS	Some coll	Coll grad	Black	White
%	7	64	29	64	21	14	49	36	15	0	21	69	12	72
AHEI score	56	61	62	61	60	58	62	60	59	55	58	62	59	61
ß (CI)	1.3 (0.7, 1.8) per 5 years			-0.9 (-0.4, -1.3) per 5 units			-1.5 (-0.8, -2.2) per child			-5.2 (-3.5, -7.0) <HS v. Coll grad			1.3 (-0.2, 2.8) Black v. White	

* ß is from multivariate model adjusted for all characteristics simultaneously
Implications: These results could be used to tailor nutritional education messages to pregnant women to avoid long term developmental sequela of suboptimal maternal nutrition.

P3-078
Early Onset of Coronary Heart Disease After Prenatal Exposure to the Dutch Famine R.C. Painter*, S.R. de Rooij*, T.J. Roseboom*, P.M.M. Bossuyt*, T.A. Simmers§, C. Osmond#, D.J.P Barker§§, O.P. Bleker## * Department of Clinical Epidemiology and Biostatistics, Academic Medical Center at the University of Amsterdam, Amsterdam, the Netherlands § Department of Cardiology, Academic Medical Center at the University of Amsterdam, Amsterdam, the Netherlands # MRC Epidemiology Resource Centre at the University of Southampton, Southampton, UK §§ Developmental Origins of Adult Disease Centre, University of Southampton # # Department of Obstetrics and Gynecology, Academic Medical Center at the University of Amsterdam, Amsterdam, the Netherlands

Background There is limited evidence that undernutrition around the time of conception is associated with an increased risk of coronary heart disease in later life. **Aim** To assess whether people who were conceived during the Dutch famine have an early onset and higher rates of coronary heart disease. **Methods** We compared the cumulative incidence of coronary heart disease among subjects exposed to prenatal famine during late (n=160), mid (n=138) and early (n=87) gestation to 590 unexposed subjects, using a Cox regression model. All subjects were born as term singletons in the Wilhelmina Gasthuis in Amsterdam around the time of the 1944-1945 Dutch famine. Coronary heart disease was defined as the presence of angina pectoris according to the Rose questionnaire, a history of coronary revascularisation or Q waves on the ECG, based on information from clinic visits at age 50 and age 58. **Results** Subjects conceived during the famine had the highest cumulative incidence of coronary heart disease (13%, hazard ratio adjusted for sex 1.9, 95% confidence interval 1.0 to 3.8 compared to unexposed). The hazard ratio was little changed by adjusting for smoking and social class. Among the 83 subjects with coronary heart disease, those conceived during the famine were 3 years younger at diagnosis. Correcting for size at birth did not attenuate the effect of early gestation famine exposure on coronary heart disease. **Conclusion** Our findings suggest that early prenatal development may be an important period in the pathogenesis of coronary heart disease, reflected in higher rates and an earlier onset of coronary heart disease among people conceived during famine. This is in line with the evidence from animal experiments, which identify the pre-conceptional and pre-implantation periods as important for later health.

P3-079
Birth Weight of Infant in Relation to Nutritional Status of Pregnant Women Pooja Sachdeva, Kumud Khanna; Indian Council of Medical Research and Institute of Home Economics, New Delhi, India

Background: An infants birth weight is critical factor in his survival, growth and development. Low birth weight is associated with death and disability. Low birth weight infants are more likely to suffer from neurological developmental problem, learning disorders, behaviour problems and lower respiratory track infection. Several demographic, social and behavioural factor are related to low birth weight. Women who have not receive early an adequate pre-natal care are more likely to deliver low birth weight. Hence the objective was to correlate the nutritional status of pregnant women with birth weight. **Sample selection:** Guru Teg Bahadur Hospital, Delhi was selected as locale and pregnant women aged 22-35 years from lower socio-economic status who came to antenatal clinic for first time for registration between July, 2004 to March, 2005 were included in the study. They were followed till delivery and infant and mother pair from the sample. **Methods used:** Anthropometric measurement like height and weight were taken. BMI, Hb, gestational age, weight gain was calculated. Questionnaires were used to assess dietary intake and energy expenditure. Information on number of iron and folic acid was calculated. **Results:** In the present study, it was found that 31% of the infant born were low birth weight. Positive and significant correlation were found between weight gain, gestational age and haemoglobin status. Parity was found negatively but not significantly correlated with birth weight. It was also found that the subjects who consume less than 50 or less iron and folic acid tablets had significantly lesser mean birth weight of infants then those who consume more than 50 tablets of iron and folic acid during pregnancy.

P3-080
Low Birth Weight and Preterm Delivery are Associated with Young Maternal Age Rural Nepal Christine P. Stewart[1], Joanne Katz[1], Subarna K. Khatry[2], Steven C. LeClerq[1], and Sharada Ram Shrestha[2], Parul Christian[1], Keith P. West, Jr.[1]
1. Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA 2. The Nepal Nutrition Intervention Project Sarlahi, Kathmandu, Nepal

Background: Pregnancy during adolescence has been observed to be associated with a number of adverse birth outcomes including preterm delivery, low birth weight, or small for gestational age infants. The nutrient demands during a pregnancy in which the mother is still growing may limit the net nutrient availability to the fetus. This research aims to study the effects of pregnancy during adolescence in a nutritionally poor environment. **Methods:** This study utilized data from two double blind cluster randomized controlled trials of micronutrient supplements during pregnancy in the rural Terai region of Nepal. In the first trial (1994-1997), communities were assigned vitamin A, beta carotene, or placebo. In the second trial (1999-2001), communities were assigned vitamin A alone (control) or with folic acid, iron-folic acid, folic acid-iron-zinc, or a multiple micronutrient. Comparable data from the two trials for women of parity 0 or 1 and of age =25 y. was combined for this analysis. Socioeconomic data, gestational age and anthropometric measurements (birth weight, length, head circumference and chest circumference) were collected on a sub-sample of all infants within 3 days of delivery and were available for 1341 infants of mothers meeting the age and parity criteria. **Results:** Differences in anthropometric or gestational age were apparent for infants of parity 0 but not parity 1 mothers. For each year of increase in age among primiparas, there were significant increases in birth weight (16g), birth length (0.10cm), head circumference (0.05cm), chest circumference (0.10cm) and gestational age (0.11wk) after adjusting for maternal factors. There was also about a 6% decreased risk of low birth weight (LBW) (OR=0.94) and an 11% decreased risk of preterm (OR=0.89). However, there was no real difference in ponderal index (PI) (0.02 kg/m^3) or the risk of small for gestational age (SGA) (OR=0.98). **Conclusions:** Younger maternal age carries an increased risk of lower birth weight, smaller infants and preterm births among women of parity 0 but not parity 1, a difference that may perhaps be explained by the lower mean age (17.9 vs. 20.0) of parity 0 versus parity 1 women.

Table 1. Linear regression coefficients and odds ratios from logistic regression per year of increasing maternal age

	Parity 0 (n=752)		Parity 1 (n=589)	
	Coef.	CI	Coef.	CI
Birth weight (kg)	0.016	0.003-0.028	-0.012	-0.026-0.002
Birth length (cm)	0.102	0.027-0.177	-0.039	-0.109-0.032
Ponderal Index (kg/m^3)	0.016	-0.100-0.131	-0.070	-0.156-0.017
Head circumference (cm)	0.054	0.010-0.099	-0.033	-0.084-0.018
Chest circumference (cm)	0.101	0.040-0.164	-0.055	-0.121-0.010
Gestational age (weeks)	0.106	0.014-0.197	-0.040	-0.090-0.010
	OR	CI	OR	CI
Low birth weight (<2.5kg)	0.936	0.877-0.998	1.031	0.955-1.112
Small for gestational age	0.981	0.918-1.047	1.014	0.940-1.093
Preterm (<37 wks)	0.894	0.821-0.974	1.036	0.941-1.141

Models control for age of the infant at the time of the birth measurements, smoking, maternal BMI in the first trimester, years of school attended, and caste

P3-081
Association Between Intake of Fish and Fish Liver Oil With Pregnancy-Delivery Complications and Babies Size at Birth Among Women of Normal Weight Before Pregnancy. Inga Thorsdottir[1], Bryndis E. Birgisdottir[1], Sveinbjorg Halldorsdottir[1], Reynir T. Geirsson[2] [1]Unit for Nutrition Research, Landspitali University Hospital & Department of Food Science, University of Iceland [2]Department of Obstetrics and Gynecology, Landspitali University Hospital, University of Iceland, 101 Reykjavik, Iceland.

Aim: To investigate the association between intake of fish and fish liver oil with pregnancy-delivery complications and size at birth among women of normal weight before pregnancy in a fishing community. **Methods:** Intake of fish and fish liver oil in full term pregnancies [n=491;80.1%] was ascertained with a validated, focused food frequency questionnaire. Maternal characteristics, information regarding pregnancy and delivery complications and the babies size at birth were collected from maternity records at the Landspitali University-Hospital in Reykjavík, Iceland. **Results:** Women consuming fish ≥6 times per month were less likely to have symptoms of pelvic joint laxity during pregnancy compared to those consuming fish < 4 times per month (p=0.023). Frequency of fish consumption was positively associated with length at birth (p=0.009) and head circumference (p=0.010). Fish liver oil was taken by 45% of the women. For those with the

highest intake (≥ tablespoon/day), the relative risk of gestational hypertension was 4.5 (CI:1.7-12.2; p=0.003) compared to those consuming less. High intake of fish liver oil was also independently associated with shorter babies at birth (p=0.036) with smaller head circumference (p=0.003). **Conclusion:** Fish consumption might be protective against pelvic joint laxity symptoms and babies size at birth increased with increased fish consumption. A very high intake of liquid fish liver oil, consisting of omega-3 fatty acids, A- and D-vitamins, seems to be associated with an increased risk of gestational hypertension as well as independently to smaller size at birth. Constituents of fish and fish liver oil might affect birth size differently depending upon the amount consumed.

P3-082

KAP Study about Health, Nutrition and Reproductive Behaviour – Community Based Evidence from the Rural and Urban Pakistan Shujaat H Zaidi, Yumna S Chaudhri, Zulfiqar A Bhutta, Department of Paediatrics, Aga Khan University

Background The limited available information on maternal nutritional status and low birth weight in Pakistan, there was an acute need for research for the development of program to improve the current situation. A series of studies was conducted to understand the magnitude, aetiology and determinants of maternal malnutrition and LBW to develop an intervention package. An initial KAP study to gauge local attitudes and perceptions regarding health, nutrition and reproductive behaviour has been conducted in two field sites Kotdihi and Bilal Colony. **Methods** Focus group discussions were the principle method of collecting data and information. **Results** Local perceptions regarding illness revolved around two basic variables physical and non-physical identification of illness. Poor women's health status in Pakistan is as much a social as medical problem. Major underlying factors were ignorance and poverty. Severe bleeding, serious complications during delivery and late access to health facility in case of emergency are the major causes kill pregnant women. Women usually get low priority for care seeking in case of illness thus depends on self-medication and home made remedies. General ignorance about women health problems and firm belief that illness is from Allah, cultural norm of shyness and modesty also restrict women to seek antenatal care and reliance on care providers. Pre-term, very thin and small babies were mentioned as low birth weight. A strong causal link between maternal nutrition and the baby's weight was established; woman's diet during pregnancy has a direct affect on the weight of the baby. All major ingredients of four food groups' viz. milk and dairy, grain, vegetables, fruits, meat are freely available. Dietary pattern do not change from house to house on the basis of ethnicity and availability but on one's outreach to all foods available depends on his income level and itemised preference of expenditures. There was a firm belief that every food has "Hot" and "Cold" hidden effects influences the diet of women during pregnancy. Discrimination in quality of foods and its distribution is a household-level manifestation of non-egalitarian attitudes e.g. women are less valuable than men, girl child is not fully human and earners in the family deserve higher honour. Women and girls share leftover and some times only licking the pot with a piece of leftover bread. There is recognition that "Taqat Ki Dawain" or micronutrient supplements help overcome malnutrition and anaemia. **Conclusion** What emerges from the above analysis is that cultural and social determinants of health and nutrition as well attitudes regarding gender play a pivotal role in influencing the health status of women. The link identified between LBW and maternal nutrition is not only important for the child's health but also provides an opportunity to actively target and improve women's own health and overall wellbeing. Interventions of micronutrient supplementation, health and nutrition education have a formal link and behavioural adjustment, which solely depends on the degree of community involvement.

Obesity & Body Composition

P3-083

Maternal Vitamin D Status Predicts Lumbar Spine Volumetric Bone Mineral Accrual in the Offspring at 9 Years Nicholas C W Harvey[1], Muhammad K Javaid[1], Pat Taylor[2], Sarah R Crozier[1], Catharine R Gale[1], Elaine M Dennison[1], Keith M Godfrey[1] and Cyrus Cooper[1]. [1]MRC Epidemiology Resource Centre, Southampton, United Kingdom and [2]Medical Physics and Bioengineering, Southampton General Hospital, Southampton, United Kingdom.

Background: We, and others, have shown that environmental influences during intrauterine and early postnatal life modify the rate of childhood bone mineral accrual. In particular, maternal vitamin D status during pregnancy has been shown to correlate with whole body bone mineral content (BMC) in the offspring at nine years. In this study we explore the maternal influences on lumbar spine volumetric bone density. **Methods:** 210 children (112 boys) were studied at nine years. Their mothers had been characterised for anthropometry, diet, and lifestyle, prospectively from early pregnancy. The children were measured at birth and nine months. Blood samples were collected from the mothers in later pregnancy and from cord blood. At nine years old, they were invited to undergo repeat anthropometry, and measurement of their bone mass, using a DXA (Lunar DPX-L) instrument. Bone mineral apparent density (BMAD) is a mathematical estimate of volumetric bone density (vBMD), defined thus: BMAD = BMC / (BA$\sqrt{}$BA), where BMC = bone mineral content, and BA = bone area **Results:** At nine years, the boys were significantly taller (p=0.01) than the girls, and had higher age-adjusted lumbar spine (LS) BMC and BA, but lower vBMD (p=0.01, <0.001, 0.02 respectively). There was no difference in bone mineral density (p=0.95). After adjustment for gestational age and gender of the child, lumbar spine BMC, BMD and vBMD were positively associated with maternal 25(OH)-vitamin D status in late pregnancy. This association was thresholded, such that mothers in the lowest fifth of the distribution had children with significantly lower bone mass at age 9 years than those in the remaining four fifths (BMC: 9% difference, p=0.007; BMD: 7% difference, p=0.002; vBMD: 6% difference, p=0.01). **Conclusions:** Maternal vitamin D status predicts lumbar spine vBMD in her offspring at age nine years. Supplementation of vitamin D for mothers who are deficient may be an important mechanism to optimise peak accrual of volumetric bone density and reduce fractures in future generations.

P3-084

Lifetime Passive Smoking Exposure is Related to the Prevalence of General and Central Obesity Amy Z. Fan[1], Marcia Russell[1], Joan Dorn[2], Maurizio Trevisan[2] [1]Prevention Research Center, Berkeley, CA 94704
[2]Department of Social and Preventive Medicine, School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY 14214

Background. Passive smoking has been related to elevated cardiovascular risk; however, information on the relationship between passive smoking and general and central obesity is lacking. **Objective.** In this study the independent contributions of lifetime passive smoking exposure to obesity (indicated by BMI) and central adiposity (indicated by waist circumference and abdominal height) were investigated among healthy adults. **Methods.** Analyses were conducted on a population-based sample from northwestern New York State which served as healthy controls for case-control studies of chronic disease (N=3496, ages 35 to 79 years). The selected sample was free of cardiovascular disease and cancer. Tobacco and alcohol use were both assessed in great detail for lifetime, during the 12 to 24 months prior to interview, and during the 30 days prior to interview. Passive smoking exposure was assessed comprehensively, including exposure at home, at work, and at other public places or social settings, for each decade of lifetime. Quintiles for each exposure category were obtained, and each respondent was assigned a score for each category based on his/her quintile of that exposure over lifetime (a higher score represents a higher exposure). A composite score was calculated by summing up the scores to represent the overall lifetime exposure level. General linear modeling was performed controlling for age, gender, ethnicity, years of education, annual family income, cigarette smoking (lifetime cigarette pack-years, current smoking status), alcohol use (lifetime adjusted total ethanol intake, current drinking status), physical activity (lifetime average hours per week on vigorous physical activity), diet (daily total calories intake, percent calories from saturated fat, for the period of 12-24 months prior to interview).
Results. It was found that lifetime exposure to passive smoking was directly and independently related to BMI (p=0.007), waist circumference (p=0.03), and abdominal height (p=0.04). **Conclusion.** This study indicated that lifetime passive smoking exposure is related to general and central obesity.

Table 1. Plasma thiols in the fasted animal	Lean	Obese			
	LC	OC	ONR	OBF	*P*
Cysteine (μM)	131± 9[a]	183 ±10[b]	176 ±14[b]	205 ± 12[b]	0.003
Homocysteine (μM)	13.1 ± 1.26[a]	12.2 ± 0.4[a]	12.6 ± 1.2[a]	18.8 ± 1.7[b]	0.007
Cysteinyl-Glycine (μM)	8.79 ± 0.29	9.43 ± 0.74	9.45 ± 0.68	7.89 ± 1.07	0.06
Glutathione (μM)	3.57 ± 0.09	3.64 ± 0.29	3.25 ± 0.29	3.60 ± 0.49	NS

P3-085

Effect of Maternal Undernutrition on Adult Offspring Hypothalamic NPY and POMC Gene Expression in the Rat Bettina A Ikenasio-Thorpe, Bernhard H Breier, Mark H Vickers, Mhoyra Fraser. National Research Centre for Growth and Development and Liggins Institute, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand.

It is increasingly recognised that some early-life determinants of obesity and metabolic disorders are fundamentally linked to neuroendocrine dysregulation. The general aim of our research programme is to investigate whether maternal undernutrition during pregnancy can influence hypothalamic appetite regulatory gene expression in adult offspring which develop obesity and metabolic disorders. In the present study, we used a model of prenatal undernutrition in the rat and applied real-time PCR on hypothalamic extracts to quantify gene expression of neuropeptide-Y (NPY, orexigenic) and proopiomelanocortin (POMC, anorexigenic). Pregnant Wistar rats were fed either *ad libitum* (AD) or 30 % of AD intake of a standard rat chow diet throughout gestation (UN). At weaning (3 weeks), female offspring from AD or UN mothers were fed either a chow or high fat diet (45% kcals as fat) ad libitum for the remainder of the study. Brains were

collected at 24 weeks and gene expression for NPY and POMC was detected in whole hypothalami using real-time PCR. Our results show a 1.5-fold increase in NPY mRNA expression and a 0.61-fold decrease in POMC mRNA expression in chow-fed offspring from UN mothers relative to offspring from AD mothers. Results of studies to examine the impact of high fat nutrition after weaning showed that in offspring from AD mothers fed a high fat diet, there was a 0.80 and 0.64-fold decrease in NPY and POMC mRNA expression respectively, relative to chow fed AD controls. Conversely, in offspring from UN mothers fed a high fat diet, there was a 1.12 increase and a 0.86-fold decrease in NPY and POMC expression respectively, relative to UN chow fed controls. This study suggests that offspring of undernourished mothers develop changes in gene expression of key hypothalamic appetite regulatory neuropeptides, including NPY. Changes in function of appetite regulatory neuropeptides may explain at least in part some of the long-term metabolic consequences observed in this experimental approach.

P3-086

Plasma Cysteine and Homocysteine in Lean and Obese Sheep David S. Gardner, Ravinder Singh[1], Michael E. Symonds, Kevin D. Sinclair[1]; Centre for Reproduction and Early Life, Schools of Human Development and [1]Biosciences, University Hospital and [1]Sutton Bonington Campus, University of Nottingham, Nottingham, UK.

Background: Prenatal protein restriction in the rat has been shown to influence methyl group metabolism in the resultant offspring. Programmed disruption of the methionine cycle leading to elevated plasma homocysteine (hcy) concentrations is an indicator of later cardiovascular disease (CVD). No study has examined methyl group metabolism in adult sheep after nutrient restriction during early life. Here we measured cysteine, hcy, glutathione and its breakdown product cysteinyl-glycine during resting conditions after 24h fasting and at 10h after a 2h feeding period. **Methods**: At day 23 of gestation 37 twin-bearing ewes were randomly allocated to receive either a control (C, 7 MJ metabolisable energy (ME)/day; n=24) or nutrient restricted diet (NR, 50% C intake, ~3.5 MJ ME/day; n=13) from days 30 to 80 of gestation. Thereafter all sheep were fed to 100% calculated ME requirement to term (12-13 MJ/day near term). Offspring were delivered spontaneously and either ewe-reared or bottle-fed (BF, n=8 from control group; 1-1.5 L/d Volac) until weaning (10-12 weeks). From weaning to 1 year of age offspring were either reared indoors with restricted activity and increased food availability to promote fat deposition (Obese controls OC, n=8; Obese nutrient restricted ONR, n=13; Obese bottle-fed OBF, n=8) or pasture grazed with unrestricted activity (Lean controls LC, n=8). Blood was sampled at 8.00am following a 24h fast. The sheep were then fed for 2h after which any residual feed was removed; and they were blood sampled again at 8.00pm. Plasma thiols were analysed by HPLC. All animal procedures were ethically approved and performed under the auspicies of the UK Animals (Scientific Procedures) Act, 1986. **Results:** In the fasted state, plasma cysteine was elevated in obese relative to lean sheep (Table 1), but hcy was increased in obese bottle-fed sheep only. A paired comparison between the fed and fasted state, regardless of group, indicated higher thiol concentrations in the fed animal (data not shown). The increase in plasma hcy 10h after feeding was greatest (37%; 17.3 μM) in the ONR group relative to the other groups (range 0-17% increase).

	Lean	Obese			
	LC	OC	ONR	OBF	*P*
Perirenal fat (g)	553 ± 93[a]	2692 ± 294[b]	2783 ± 197[b]	3661 ± 214[c]	0.000
Pericardial fat (g)	82 ± 8[a]	328 ± 29[b]	232 ± 29[b]	341 ± 54[b]	0.000
Omental fat (g)	785 ± 95[a]	4222 ± 154[b]	3598 ± 201[b]	3788 ± 316[b]	0.000
%a perirenal (%a total)	38.1 ± 2.3[a]	36.6 ± 2.8[a]	41.8 ± 1.6[ab]	47.4 ± 2.3[b]	0.01
%a pericardial (%a total)	6.9 ± 1.4	4.5 ± 0.3	3.7 ± 0.6	4.3 ± 0.6	0.07
%a omental (%a total)	54.9 ± 2.6[ab]	58.8 ± 2.7[b]	54.4 ± 1.5[ab]	48.2 ± 2.5[a]	0.03
Subcut fat (11th rib)	4.3 ± 1.0[a]	21.5 ± 4.4[b]	22.1 ± 1.5[b]	29.2 ± 2.5[b]	0.000

Key: Values within a row not sharing a superscript are significantly different at P<0.05 (1-way ANOVA). **Conclusions:** Bottle-fed obese sheep have higher resting plasma hcy concentrations relative to other obese and lean sheep. Plasma hcy concentrations greater than 15 μM in humans predict increased risk of CVD. Increased plasma cysteine concentrations in obese relative to lean sheep suggests a predisposition to remove hcy from the circulation via the trans-sulphuration pathway in this physiological state. In conclusion, bottle feeding of sheep from birth to natural weaning specifically affects methyl group metabolism in young adult sheep during resting conditions. *(Funded by the British Heart Foundation, and the University of Nottingham).*

P3-087

Human Neonatal Body Composition is Related to Umbilical Venous and Fetal Liver Blood Flows Independently of Placental Size Guttorm Haugen,[1] Nick Harvey,[2] Cyrus Cooper,[2] Sarah Crozier,[2] Mark Hanson,[1] Hazel Inskip,[2] Torvid Kiserud,[3] Keith Godfrey[1,2] & the Southampton Women's Survey Study Group. [1]Centre for Developmental Origins of Health and Disease & [2]MRC Epidemiology Resource Centre, University of Southampton, Southampton, UK; and [3]Institute of Clinical Medicine, Department of Obstetrics & Gynecology, University of Bergen, Norway.

Background: A major proportion of the umbilical venous blood coming from the placenta perfuses the fetal liver parenchyma before reaching the systemic circulation of the fetus. This proportion is related to maternal body composition; maternal slimness is correlated with an increase in fetal liver blood flow ("liver sparing effect").[1] Umbilical venous and fetal liver blood flows are both positively related to neonatal weight, but the relations with neonatal body composition are unknown. **Methods**: We studied 152 singleton uncomplicated pregnancies in the Southampton Women's Survey (SWS), a population survey of women characterised before and during pregnancy. At a median (10^{th} – 90^{th} centile) gestation of 36^{+0} weeks (35^{+1} – 37^{+1}) we derived umbilical venous and ductus venosus blood flows using ultrasound Doppler technique (Sequoia, Acuson, Mountain View, CA). We measured internal vessel diameter (D) (mean of 5–10 measurements) and time-averaged maximum velocity (TAMX). Umbilical vein and ductus venosus flows were calculated as $(D/2)^2 \cdot p \cdot TAMX \cdot h$ where D is the vessel diameter, and h the coefficient for the spatial blood velocity profile (umbilical vein = 0.5; ductus venosus = 0.7). Liver blood flow was calculated as umbilical venous – ductus venosus flow. Neonatal body composition (bone mineral content, lean mass and fat mass) was measured by DXA scan (Lunar DPX-L, US) and expressed as weight (bone mineral content and lean mass), z-score (fat mass) or as percentage of birth weight. The z-scores were derived from data in the SWS (n=614). **Results:** Higher umbilical venous and liver blood flows were both highly correlated (P<0.0001) with neonatal bone mineral content (r=0.39, r=0.31, respectively), lean mass (r=0.43, r=0.39, respectively) and fat mass (r=0.52, r=0.44, respectively). The increases in fat mass with higher umbilical venous and liver blood flows were greater than those in bone and lean mass; in consequence, higher umbilical venous and liver blood flows were strongly associated with a greater %body fat (r=0.47 and 0.40, respectively, both P<0.0001). Larger placental weight was associated with greater bone, lean and fat mass (r=0.59, 0.54 and 0.61, respectively, all P<0.0001) and with greater liver blood flow (r=0.39, P<0.0001). Simultaneous analyses taking account of placental size showed that, independently of placental weight, higher liver blood flow was not associated with neonatal bone mineral content (P=0.15) but was associated with greater lean mass (P=0.001), fat mass (P=0.002) and %body fat (P=0.009). **Conclusions:** Our findings suggest a major influence of umbilical venous and fetal liver blood flows on neonatal body composition, with much of this influence acting independently of placental size. Higher umbilical venous and fetal liver blood flows are associated with a greater increase in fetal fat mass than in bone or lean mass. These findings have important implications for strategies to reduce the prevalence of obesity in later postnatal life. The British Heart Foundation and The Research Council of Norway supported the study. [1]Haugen et al. *Circulation Res* 2005;96:12-14.

P3-088

Birth Weight, Adjusted for Gestational Age, is Inversely Associated with Central Fat Mass Daniel J. Hoffman[1], Meredith S. Dolan[2], John D. Sorkin[3]; [1]Rutgers, the State University of New Jersey, Department of Nutritional Sciences, New Brunswick, NJ; [2]University of Pennsylvania School of Medicine, Department of Psychiatry, Philadelphia, PA; [3]University of Maryland School of Medicine, Division of Gerontology, Baltimore VA Medical Center, Baltimore, MD

Background: The relationship between undernutrition in utero and risk for chronic diseases in adulthood is supported by several animal and human epidemiological studies, but few provide clear mechanisms to support a physical causal pathway. Abnormal body fat distribution in children who were born growth retarded may be part of this causal pathway, but relatively few studies have used precise measures of body composition, leading to equivocal and sometimes contradictory results. Thus, the objective of this project was to determine the realationship between birth weight and adiposity in a large cohort of children using a precise measure of body composition. **Methods:** Subjects included 101 children who underwent dual x-ray absorptiometry (DXA) scans between 1993 and 1998. Birth weight was determined by maternal recall. Multiple linear regression analysis was used to determine the relationship between birth weight, adjusted for gestational age, and the following three outcome variables: total fat mass, truncal fat mass, and percent body fat. Confounding variables, race, sex Tanner stage, and current body weight, were included in each of the regression models. **Results:** The mean age of the children studied was 12.9 ± 2.4 years and the mean birth weight reported by subjects' mothers was 3.3 ± 0.5 kg. The total fat and truncal fat mass was 12.8 ± 8.7 kg and 5.1 ± 4.1 kg, respectively, and the mean percent body fat was 22.9 ± 10.3 %. Birth weight, both unadjusted and adjusted for gestational age, was not a significant predictor for any measure of body fatness. However, birth weight adjusted for gestational age was a significant (p=0.0178) negative predictor of truncal fat mass, adjusted for total fat mass, independent of race, sex, Tanner stage, and current weight. **Conclusions:** While birth weight adjusted for gestational age was not associated with total or truncal fat mass or percent body fat, it was inversely associated with truncal fat mass when adjusted for total fat mass, independent of race, sex, Tanner stage, and age.

P3-089

Dietary Fructose during Suckling Increases Body Weight and Fatty Acid Uptake into Skeletal Muscle in Adult Rats Minh Huynh[1], Joost JJP Luiken[2], Will Coumans[2], Rhonda C. Bell[1];[1]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, [2]Dept of Molecular Genetics, Maastricht University, 6200 MD Maastricht, The Netherlands

Background: Dietary intake of fructose has increased substantially in the past 3 decades, but the health consequences of these changes have not been fully described. In weanling rats, consumption of a high fructose diet results in insulin resistance within a few weeks. Whether metabolic perturbations resulting from such dietary intake persist beyond the time of fructose consumption, thereby inducing metabolic programming, is unknown. In other models of diet-induced

insulin resistance, increased fatty acid uptake rates into skeletal muscle has been observed This study examined the effects of consuming fructose during the suckling period on fatty acid uptake in skeletal muscle, adipose and liver tissue. We also measured bodyweight and other metabolic parameters in adult rats. **Materials & Methods:** Rat pups were artificially reared from 12 – 19 days of age. Suckling diets consisted of: 1) LAC (lactose as the sole carbohydrate, 2) FR (1:1 lactose:fructose), 3) GAL (1:1 lactose: galactose). Total carbohydrate content of all diets was equal. A fourth group remained with a dam as the suckle control group (SC). Pups were fed a purified rat chow from 21 to 77 days of age. To examine the effects of feeding a high fructose diet in adult life, half of the SC group was fed high fructose chow (65% kcal as fructose) from 56 – 77 days (LFr). Body weight and food intake were measured weekly, blood (non-fasting) was drawn bi-weekly for determination of insulin & glucose. At 77 days of age, epidydimal fat pads were removed and weighed. Fatty acid uptake into skeletal muscle (hind limb), liver or retroperitoneal fat pads was measured using giant membrane vesicles prepared from these tissues. **Results:** Bodyweight of FR rats at 11 weeks of age was higher than those in the SC and LFr groups (557 ± 22, 483 ± 22, 452 ± 22 g respectively) ($p< 0.05$ for both). Food intake did not differ between groups throughout the study. FR rats had heavier fat pads compared to LFr (12.4 ± 1.2 vs. 7.5 ± 1.2 g) ($p< 0.05$). Fatty acid uptake into vesicles made from muscle was higher in the FR group than in the SC group (17.6 ± 2.6 vs. 11.0 ± 0.8 pmol/mg protein/15s) ($p< 0.05$). Insulin concentrations were higher in the FR rats compared to LFr at 56 days (0.52 ± 0.10 vs. 0.17 ± 0.09 ng/ml) and at 70 days (0.58 ± 0.12 vs. 0.18 ± 0.11 ng/ml) ($p< 0.05$ for both). Glucose concentrations were similar among all groups except at 77 days, when LFr rats had higher glucose levels than FR rats (177 ± 4 vs. 165 ± 4 mg/dl) ($p< 0.05$). **Conclusion:** Introduction of fructose during the suckling period can lead to elevated fatty acid uptake into skeletal muscle in adulthood. This is accompanied by increased circulatory insulin levels and increased bodyweight. Exposure to fructose in early life may contribute to programming of insulin resistance and the associated sequelae in adulthood.

P3-090

Early Determinants of Body Fat in 17 y Old Adolescents from a Cohort of Healthy Term Danish Infants Helga K. Ingstrup, Anni Larnkjaer, Lene Schack-Nielsen, Christian Mølgaard, Kim F. Michaelsen; Center for Advanced Food Studies, Department of Human Nutrition, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark.

Background The prevalence of obesity both among children and adults is increasing worldwide. Several studies have shown an association between rapid early weight gain on risk of obesity as adult in both preterm and term infants. These studies used BMI to classify obesity, but body fat percent is a more accurate measurement of obesity, since BMI also reflects lean body mass. **Purpose:** To examine the effect of infancy weight gain in healthy term infants on body fat percent in adolescence. **Methods:** 143 healthy, term Danish infants participated in a prospective observational study (*The Copenhagen Cohort Study on Infant Nutrition and Growth*). Only children with a birth weight between the 10th and 90th centile were included. Measurements at birth and at 9 months were used in this analysis. Follow-up at the age of 17 is ongoing but most of the children have been examined including anthropometric measurements, and information on body composition from whole body DEXA-scan (Hologic 1000/W). All subjects for whom data existed on both weight at birth and 9 mo and DEXA-scan at age 17 y were included in this analysis (n= 92 (male = 38; female = 54). The association between birth weight and weight gain from birth to 9 mo and body fat % was examined by linear regression with gender as a fixed factor. **Results:**. 7 % and 1 % of the participants were overweight or obese, respectively (Cole et al. BMJ 2000). The mean (SD) body fat percent was 13.5 % (4.4) for boys and 25.9 % (4.5) for girls. There was no significant effect of birth weight, but a significant effect of weight gain ($p= 0.007$, 1 % increase in body fat percent for each kg increase in weight gain from birth to 9 mo). The effect of gender was highly significant ($p< 0.001$) with a 13.7 % point higher fat % in girls. **Conclusions:** Weight gain during the first 9 months of life in healthy Danish term infants is positively related to body fat percent in adolescence, independent of birth weight. Further analysis will include dietary data and skin fold measurements during infancy and an analysis of predictors of regional fat distribution at age 17 years.

P3-091

Is Birth Weight Associated with Grip Strength in Young Women? Findings from the Southampton Women's Survey Hazel M. Inskip, Keith M. Godfrey, Helen J. Martin, Cyrus Cooper, Avan Aihie Sayer & The Southampton Women's Survey Study Group. (MRC Epidemiology Resource Centre, University of Southampton, Southampton SO16 6YD, UK)

Background: Consistent relationships between grip strength and birth weight have been found in people over 50 years of age[1-3]. Grip strength is a measure of muscle function and these results suggest that factors influencing growth and development in utero have lasting consequences for muscle strength in later life. Little is known, however, about whether these relationships are apparent at younger ages. **Methods:** The Southampton Women's Survey is a follow-up study of over 12,500 women aged 20-34 years from Southampton UK. We interviewed the women in their own homes when they were not pregnant to obtain data that included measurement of height using a portable stadiometer (Harpenden, CMS Weighing Equipment Ltd., London) and details about the women's physical activity. We also asked the women recall their own birth weight. Those women who subsequently became pregnant have been followed through their pregnancy, and the offspring studied in early life. At 19 weeks of pregnancy, we measured grip strength using a Jamar handgrip dynamometer. Three readings were taken for each hand, and the highest of the six values was used in the analysis. Multiple linear regression was used to relate grip strength measurements to the women's age, height, exercise levels, and their own weight at birth. **Results**: 682 women who were interviewed before pregnancy had their grip strength measured during pregnancy. Their age at grip strength measurement ranged from 20 to 38 years. Mean grip strength was 32.5 kg (range 10 to 52 kg). Grip strength increased with age by 0.2 kg per year of age, (95%CI: 0.08 to 0.3 kg) and by 0.4 kg for each centimetre increase in height (95%CI: 0.3 to 0.5 kg). Among those who did at least one hour of strenuous exercise per week, grip strength was 2.1 kg higher (95%CI: 1.0 to 3.1 kg) than in those who at recruitment interview reported doing no strenuous exercise in the previous three months. Grip strength was also related to birth weight in the 495 women who were able to recall their own weight at birth; after adjustment for age, height, and exercise, grip strength increased by 1.2 kg for each kilogram increase in birth weight (95%CI: 0.5 to 2.0 kg). **Conclusion**: We have shown a positive association between weight at birth and grip strength in young women, independent of their age, height and current level of exercise. This is consistent with the findings from previous studies of older people. These results suggest that birth weight and its determinants are related to peak muscle strength attained in young people as well as to declining muscle strength in later life. Poor muscle function in older life has a major impact on quality of life. Improving the early environment may thus have a beneficial effect on muscle function in older people by maximising the peak levels they obtain as young adults and minimising the rate of decline in later years. References: [1]Sayer AA *et al.* Age & Aging 1998;27:579-583. [2]Kuh D *et al.* Am J Epidemiol 2002;156:627-633. [3]Sayer AA *et al.* J Gerontol 2004;59A:930-934.

P3-092

The Premature Leptin Surge –The Key of Developmental Origins of Obesity Hiroaki Itoh, Shigeo Yura, Norimasa Sagawa, and Shingo Fujii; Department of Gynecology and Obstetrics, Kyoto University Graduate School of Medicine, Kyoto, Japan, 606-8507. Department of Obstetrics and Gynecology, School of Medicine Mie University, Tsu, Japan.

Objectives: Undernutrition in utero is closely associated with obesity relating to detrimental metabolic sequelae in adulthood. We established a mouse model in which offspring with fetal undernutrition (UN offspring: 30% reduction of maternal food intake) on a high-fat diet (HFD) develop pronounced adiposity (30% increase in fat deposit). In the neonatal catch-up period, UN offspring exhibited a premature surge of leptin (8-10 days after birth), compared to that (16 days after birth) of offspring with intrauterine normal nutrition (NN offspring). To investigate a possible involvement of "premature leptin surge" in the developmental origins of obesity, we developed an animal model of artificial premature leptin surge by neonatally leptin administration (6-11 days after birth; NN-Lep offspring). **Meythods:** Immunohistochemistry of neuropeptide Y (NPY) was done in arcuate nucleus (ARH) and in paraventricular hypothalamus (PVH). Measurement of decrease in calorie intake and body weight loss (12 hours), Western blot analysis of phosphorylated signal transducer and activator of transcription 3 (STAT3) in the entire hypothalamus (30 min) and immunohistochemistry of c-Fos in ARH as well as in PVH (135 min) were carried out after acute administration of leptin. The identical fetal undernutrition experiment was carried out using leptin deficient *ob/ob* mice. **Results:** On a HFD, NN-Lep offspring developed pronounced obesity (30% increase in fat deposit) compared to vehicle-treated groups. Both UN offspring and NN-Lep offspring exhibited impaired responses to leptin administration in decrease of calorie intake as well as body weight loss, in the hypothalamic phosphorylation of STAT3, and in detection of c-Fos positive cells in ARH as well as PVH. Thus, both NN-Lep offspring, with artificial "premature leptin surge", and UN offspring showed undistinguishable obesity prone phenotypes with similar leptin resistance, strongly suggesting a pivotal role of "premature leptin surge" in the developmental origins of obesity. Moreover, undernutrition in utero did not cause augmentation in HFD-induced obesity in leptin deficient *ob/ob* mice offspring, indicating that leptin is inevitable in the regulation of the developmental origins of obesity. **Conclusions:** "Premature leptin surge" plays a critical role in the developmental origins of obesity.

P3-093

Status of Zinc, Copper, Magnesium, Iron, Folic Acid, and Iodine amongst Young Adult Women in a Rural Block of Haryana State, India Umesh Kapil, Priyali Pathak, Department of Human Nutrition ; All India Institute of Medical Sciences ,New Delhi , India

The incidence of low birth weight (LBW) babies in developing countries is in the range of 30 – 40%. Deficiencies of micronutrients during pregnancy have been documented in causation of LBW. Studies have been undertaken to study the micronutrient(s) status amongst pregnant women. Data on the serum levels of micronutrients amongst young adult women (parity nil) is limited hence, the present study was undertaken to assess the status of zinc, copper, magnesium, iron, folic acid and iodine amongst young adult women (YAW) in a rural area. A community based cross sectional survey was conducted. Two hundred and eighty eight YAW aged 18 years and more were enrolled. The study was conducted

during November 2000 and October 2001. Data was collected on socio-economic status and other demographic parameters. Blood was collected, serum separated, and analyzed for the micronutrient by standard methods. The urine sample was analyzed for iodine by the wet digestion method. Nearly 41.5, 28.2, 18.2, 63.8, and 27.7 percent had zinc, copper, magnesium, iron, and folic acid deficiency, respectively. No woman had iodine deficiency. The findings of the present study reveal that a high prevalence of micronutrient deficiencies exists amongst the YAW in the rural area studied. We suggest that further studies are needed to provide more information on the data on micronutrient deficiencies amongst young women from various part of the country, so that we may develop supplementation strategies of the micronutrients, if required.

P3-094

Determinants of Obesity in Pune Urban Children – Increased Food Intake or Decreased Physical Activity? Vaishali A Madkaikar, Anjali S Mote, Madhumati S Otiv, Sheila A Bhave, Ashish R Bavdekar, Anand N Pandit, Department of Pediatrics, KEM Hospital, Pune 411011, India

Background: Obesity epidemic is global. Developing countries have double burden of malnutrition and obesity in children. There is a clear divide between rural and urban prevalence of obesity, probably related to changing eating habits (more junk & less fibre foods), reduced physical activity and obesogenic schools. **Objective:** This study was designed to assess the dietary intake and physical activity in 16-17 year old urban Indian children and its effect on adiposity. **Methodology:** 141 children (70 girls and 71 boys) from Pune urban cohort were studied at mean age of 16.5 yr (SD =0.66). A detailed anthropometry (weight, height, waist circumference, skin-folds) was done during their visit. Sum of skin-fold (SSF) was the sum of subscapular, biceps, triceps and suprailiac skinfold. Dietary assessment was done by one-day recall, two-day diary method and food frequency questionnaire. Dietary data was validated in 20% children using food weighment method. Physical activity was assessed by one day physical activity recall. Daily energy expenditure was calculated using physical activity ratios and estimated BMR using standard methods (PAR / MET values). Socio economic status was assessed by Kuppuswamy scales. To calculate the calories, protein and fat Indian reference standard was used. **Results:** The mean calorie intake was 1720 (SD 476) Kcal (80% of RDA). The mean protein intake per day was 49 gm (SD14.2), and fat intake was 47gms (SD18.0). 61 (43%) children received more than 25% of calories from fat (RDA < 25% from fat). 63% children consumed less than recommended (RDA 3 / day) servings of vegetables and 73% children consumed less than recommended (RDA 2 /day) servings of fruits. Mean calorie (r 0.34 p= 0.00), protein (r 0.29 p=0.00), and fat (r 0.40 p=0.0) consumption showed significant direct correlation with SES but not with BMI, skinfold thickness and waist circumference. However vegetable serves per day correlated inversely with subscapular skinfold (r -0.17, p= 0.05). Frequency of consumption of salad had inverse correlation with BMI (r -0.21, p=0.01), weight (r -0.18, p=0.03), waist circumference (r -0.23, p=0.01), subscapular skinfold (r -0.20, p=0.02) and also the sum of skinfolds (r -0.17, p=0.042). Intake of meat (r 0.177, p=0.04), colas and refined foods (r 0.17, p=0.04) directly correlated with subscapular skinfold. The recommended moderate activity of at least 30 minutes was seen only in 28% of children. (Boys 32.4%, Girls 24.2%) Increased exercise hours correlated inversely with sum of skinfolds (r –0.26, p=0.00) and with waist circumference (r -0.29, p=0.001). Mean time spent on TV hrs was 2.3 hrs (SD 1.9) which had direct correlation with BMI (r 0.236, P= 0.002), weight (r 0.180, P= 0.033) and SSF (r 0.215, P=0.011). These 141 children came from 36 school and junior colleges and the results showed that 83% of institutes had physical activity period-less than 3 times a week. Multivariate analyses of the covariates showed that excess TV hrs (ß =0.184) and less exercise hours (ß = -0.240) predicted higher waist circumference (R^2=9.0%). Determinants of BMI were similar to that of waist circumference. **Conclusions:** Although the caloric intake was appropriate in 78% the energy came from excess amount of fat. Fruits and vegetables intake and physical activity was grossly inadequate in 60 -70% of children. However excess sedentary habits remained the most important determinant of central and overall adiposity.

P3-095

Is China Facing the Epidemic of Obesity and Cardiovascular Risk Factors in Children and Adolescents? Jie Mi, Dongqin Hou, Hong Cheng Tianyou Wang, Xiaoyuan Zhao, Xiuyuan Ding, Xiaoyi Shan, Li Zhang; Department of Epidemiology, Capital Institute of Pediatrics, Beijing 100020, China

Background The prevalence of obesity in children and adolescents is increasing rapidly worldwide. This raises concerns about the accompanying cardiovascular risk factors including hypertension, dyslipidaemia, hyperglycemia, which are usually clustered to one individual and leads to an increased likelihood of cardiovascular disease in adulthood. So far, no large-scale representative samples have been studied to present the actual status of obesity and related cardiovascular risk factors among children and adolescents in China. **Methods** A cross-sectional survey with Beijing, the metropolis representative sample of 21 902 children and adolescents (aged 3-18 years, male 11 045, female 10 857) was conducted during April to October, 2004. Blood pressure (BP), weight and height were measured for all participants. The fourth Korotkoff sound (K4) was as the definition of diabolic BP (DBP). Fasting finger-stick capillary samples were used to assess the concentration of glucose, total cholesterol (TC) and triglyceride (TG) by ACCUTREND GCT (Roche Diagnostics Shanghai Limited) for 20 638 schoolchildren (aged 6-18 years, male 10 411, female 10227). The overweight and obesity were defined using the Chinese sex- and age-specific body mass index cutoffs for schoolchildren aged 7 to18 years. Owing to the above Chinese BMI reference norm don't cover the children population under 7 years, overweight and obesity for children aged 3 to 6 years were defined by the sex- and age-specific body mass index cutoffs recommended by NCHS/CDC (USA, 2000). Pre-hypertensive (also termed "high normal") and hypertension were defined using the Fourth Report on High Blood Pressure in Children and Adolescents (USA 2004) . Hyperglycemia (glucose >=6.1 mmol/L), hyperlipidemia (total cholesterol >=5.2 mmol/L and/or triglyceride >=1.7 mmol/L) were diagnosed accordingly. **Results** The overall prevalence of overweight and obesity was 11.8% and 9.7% (boys: 14.1% and 12.4%; girls: 9.5% and 7.0%) for the whole sample population, respectively. The age-specific prevalence was 9.1% and 6.9% for the preschool (aged 2 to 5 years), 11.1% and 11.6% for the primary school (aged 6 to12 years), 12.8% and 9.6% for the junior high-school (aged 13 to 15 ages) , 12.6% and 6.5% for the senior high-school (aged 16 to18 ages), respectively. Children who resided in urban presented were heavier than those in the rural (urban vs rural: overweight 14.1% vs 9.0%; obesity 12.2% vs 6.8%). The overall prevalence of pre-hypertensive and hypertension for the whole sample population was 9.8% and 5.1%, respectively, similar to the predicted 10% and 5% prevalence of pre-hypertensive and hypertension in children and adolescents in the United States. The highest rates were found in the group of preschool children (17.9%, 7.7%), similar levels in the primary school (9.3%, 5.2%) and junior high-school (9.8%, 5.4%), the lowest in the senior high-school (8.6%,4.1%). In contrast to the distribution of overweight and obesity between urban and rural, children in the rural presented more frequencies of pre-hypertensive (10.8%) and hypertension (6.0%) than children in the urban (8.8%, 4.4%) (x^2=61.8, P<0.001). Hyperglycemia, hyperlipidaemia were presented in 0.7%, 9.1% of schoolchildren (aged 6 to18 years). Hypertension was the most prevalent component of MS in obese children, more than one-third (39.5%) of the obese presented with pre-hypertensive and/or hypertension, which gave 3.9 times as high as that for the control group (no-overweight and/or no-obesity). The prevalence of hyperglycemia and hyperlipidaemia in obese children were 1.2% and 24.6%, respectively, with 2.0 times and 3.5 times as high as that for the control group. **Conclusions** Hypertension, obesity and hyperlipidaemia, the risk factors for MS, were not confined to the industrialized countries. The high rates have been already evident in the urban of developing countries such as Beijing of China.

P3-096

Increased Maternal Nutrition Increases Hypothalamic Expression of the Appetite Inhibitor Proopiomelanocortin in the Postnatal Lamb BS Muhlhausler[1], CL Adam[2], PA Findlay[2], JA Duffield[1], IC McMillen[1]; [1]Research Centre for the Early Origins of Adult Health, School of Molecular and Biomedical Science,The University of Adelaide, Australia; [2] Energy Balance and Obesity Division, The Rowett Research Institute, Aberdeen, UK

Introduction: Epidemiological studies have demonstrated that infants of diabetic mothers, who are exposed to an elevated nutrient supply during development, are at increased risk of altered appetite regulation and obesity in later life. In the present study we have therefore determined the effect of increasing maternal nutrient supply on components of this appetite-regulating neural network in the early postnatal period. **Methods:** From 115 d gestation until delivery pregnant ewes were fed a diet which provided either 100% (control, n=12) or ~150% (Well Fed, n=9) of maintenance energy requirements (MER). Lambs were delivered spontaneously and birth weight was recorded within 6 h of birth. Blood samples were collected every 72 h for the determination of glucose, insulin and leptin concentrations. On postnatal day 30, postmortem was performed and brains were collected. *In situ* hybridisation was then used to determine the expression of OBRb, the appetite-stimulating neuropeptides Neuropeptide Y (NPY) and Agouti-related protein (AGRP) and appetite-inhibiting neuropeptide precursor Proopiomelanocortin (POMC) and neuropeptide Cocaine-amphetamine-regulated transcript (CART) in the hypothalamic arcuate nucleus. **Results:** Maternal nutrient intake between 115 d gestation and delivery was higher in the Well Fed group (129 ± 4 % vs 90 ± 2 % MER, P<0.001). Plasma concentrations of glucose, but not insulin or leptin, were significantly higher in lambs of Well Fed ewes compared to Controls (F=5.93;P<0.05). Hypothalamic expression of the appetite-inhibiting neuropeptide precursor POMC was higher in the Well Fed group (0.48 ± 0.09 vs 0.28 ± 0.04, P<0.05) and tended (P=0.07) to be positively correlated with plasma glucose concentrations when data from all lambs were combined. CART mRNA expression was positively correlated with plasma glucose, insulin or leptin concentrations in Control, but not Well fed lambs. NPY and AGRP were inversely correlated with total relative fat mass in both the Control and Well Fed groups (NPY, r=0.53 P<0.04; AGRP, r=0.63 P<0.01). In the Well Fed group, but not Controls, OBRb expression was inversely related to relative subcutaneous fat mass (R=-0.74; P<0.05, n=8). There was no difference in hypothalamic expression of CART, NPY, AGRP or OBRb mRNA between the Control and Well fed groups. **Conclusion:** We have therefore demonstrated that increasing maternal nutrient intake in late gestation results in changes to the network which regulates appetite in postnatal life. This provides evidence that important components of the appetite regulating system may be programmed by exposure to increased nutrient supply before birth.

P3-097

Developmental Exposure to Environmental Estrogens is Associated with Obesity Later in Life Retha R. Newbold, Elizabeth Padilla-Banks, Ryan Snyder and Wendy N. Jefferson; Laboratory of Molecular Toxicology, National Institute of Environmental Health Sciences (NIEHS), NIH, DHHS, Research Triangle Park, NC 27709

Background: Obesity is a significant human health problem that has reached epidemic proportions over the last 2-3 decades. Obesity and overweight are associated with increased risk of Type 2 diabetes, insulin resistance, coronary heart disease, high blood pressure, stroke, gout, liver disease, asthma and pulmonary problems, gall bladder disease, kidney disease, reproductive problems, psychological and social problems, osteoarthritis, and certain cancers. Obesity is caused by a complex interaction between genetic, behavioral and environmental factors. Commonly held causes of obesity are overeating and a sedentary lifestyle imposed on a background of genetic predisposition for the disease. Although much interest has focused on these factors, the etiology of obesity remains uncertain. A recently emerging hypothesis is that in utero and early developmental exposure to environmental chemicals plays a role in the development of obesity later in life. For years, research in our laboratory has focused on the effects of estrogenic compounds on differentiation. Our working premise is that the developing organism is extremely sensitive to perturbation by chemicals with estrogenic or endocrine disrupting activity, and that exposure to these chemicals during critical stages of differentiation may have permanent long lasting consequence. Using diethylstilbestrol (DES) as a model estrogenic chemical, our major focus has been on abnormal reproductive tract differentiation. However, we have also examined the effects of DES and other environmental estrogens including genistein, a phytoestrogen found in soy products, on body weight. **Methods:** CD-1 mice were treated with DES (0.00001, 0.001 and 1 mg/kg/day) or genistein (50 mg/kg/day) on days 1-5 of neonatal life. Body weights were recorded daily during treatment and until weaning; thereafter, weights were determined weekly for 14 weeks and once per month until sacrifice. **Results:** Body weights were not different between untreated control and DES-treated mice during treatment; however, at 6 weeks of age, DES treated mice exhibited a statistically significant increase in body weight which was associated with an increase in % body fat. Using PIXImus^{TM} mouse densitometry, we measured the % fat mass in neonatal DES treated mice at 6 weeks of age and showed a significant increase over untreated controls. Neonatal exposure to genistein also caused a significant increase in body weight suggesting that DES is not unique in causing these effects. Fat depots (retroperitoneal, inguinal, parametrial, gonadal and brown fat) from DES mice were collected and weighed to determine alterations in adipose tissue including size of specific fat pads. Serum hormone levels for leptin, adiponectin, IL6, insulin, free fatty acids, glycerol, and triglcerides were also measured. Interestingly, measurements of activity and food intake were not different between control and treated mice. **Conclusions:** Taken together, our data supports the idea that brief exposure to low levels of environmental estrogens early in life is associated with increased body weight as the mice age. Whether our results can be extrapolated to humans remains to be determined but it provides a fruitful area of further research.

P3-098

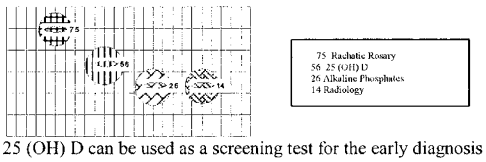
Assessment of Intergenerational Differences in Food Habits of Families Seenu Raj, India

Intergenerational differences in food habits of families comprising three Generations with woman gainfully employed outside the home were studied. A total of 30 high income group families in Faridabad were selected by purposive sampling technique comprising a total number of 148 respondents. The tools used in the study were Questionnaire cum interview schedule, 24 hours dietary recall, food frequency questionnaire and anthropometry. A study of food expenditure pattern of the families revealed that the major contributors to the food expenditure were milk and milk products (15.71%), eating out and food ordered (16.58%), snacks (9.38%) and convenience food (7.97%). Majority of the elderly (67.4%) were vegetarians whereas non-vegetarianism was more pronounced in second and third Generation. Menu of elderly was observed to be woven around traditional foods whereas that of middle Generation was deviating away from the traditional foods. Children showed the most deviation from traditional to westernized culture. Increased consumption of soft drinks was found to replace milk from the diets of children. Frequency of eating out and fast food intake such as pizza, burger, chowmein, french fries etc was high in middle Generation and children. Overall nutrient intake of Generation 2 was observed to be the most adequate with respect to all the nutrients, whereas Generation 1 was seen to be inadequate in energy, protein and iron. As the age of children increased, adequacy decreased with respect to energy, protein, Vitamin A and iron, where as percentage contribution of fat to energy increased. The percentage energy available from fat was 38.92% in children followed by 34.84% in Generation 2 and 31.75% in elderly. Anthropometric measurements of children revealed that 24.4% children were at risk of being overweight and 11% were overweight. Difference in food habits were observed across the three Generations. The range of foods consumed by young generation is moving away from traditional foods to more western pattern of food consumption leading to increased prevalence of overweight in children.

P3-099

Fetal Origins of Osteoporosis L.G.RAMAVAT Ahmadi Hospital, Kuwait. Vingta No.3, Kistoo Lane, Vacoas, MAURITIUS drlgramavat@yahoo.co.in; ramavat@fetaloriginsofosteoporosis.com

Several fullterm newborns weighing more than 2.5 kg are born with vitamin D deficiency rickets, due to insufficient calcium transfer from mother to fetus. If undiagnosed these newborns will suffer from Osteoporosis later in life. Author examined 858 newborns within 24 hrs of their birth in the neonatology unit of the pediatric department of Ahmadi Hospital, Kuwait, over a period of two years. Apart from the routine examination these newborns were specifically examined for rachitic rosary, widened anterior/posterior fontanelle, sagittal suture and hypotonia. Gestational age and maturity was assessed by Dubowitz method. Venous blood was obtained and three to five ml of serum was sent on the same day by air to J.S. pathology PLc, London for the estimation of 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol by Radio Immuno Assay. X-ray of the wrist, calcium, phosphorus and alkaline phosphates were done in our hospital's laboratory. 75 newborns out of 858 fullterm, normal delivery and weighing more than 2.5 kg were born with rachitic rosary. 25-hydroxycholecalciferol was lower than normal in 56 newborns (mean:12.5;range:3-22nmol/L) and normal (>25nmol/L). Calcium concentration was within the normal range in all 56 newborns (2.2-2.62-mmol/L). Plasma phosphate concentration was significantly higher in all the newborns (mean:1.7;range:1.6-1.8mmol/L) and normal (mean:1.5;range:0.81-1.58mmol/L). Plasma alkaline phosphates was higher than normal in twenty six newborns (mean195; range: 170-220u/L) and normal (mean195; range: 170-220u/L) for our laboratory. Fourteen newborns had the radiological changes, early flaring, widening and cupping seen in their wrist x-ray.



25 (OH) D can be used as a screening test for the early diagnosis of osteoporosis. The seeds of osteoporosis are sown early in life, lack of intake of calcium and vitamin D in adequate quantities and an insufficient amount of weight bearing exercise (walking and running but not swimming) leads to failure in achieving peak bone mass in last adolescence and early adulthood. An adequate amount of vitamin D, throughout one's life (in conjunction with exercise, proper nutrition, calcium and magnesium) is necessary for preventing bone loss. Low levels of vitamin D and insufficient sunlight exposure (less than 20 minutes per day) are associated with osteoporosis. Vitamin D is needed to properly absorb calcium. Calcium, together with vitamin D, can help heal bone fractures from osteoporosis and decreases the risk of future bone breaks

P3-100

Acute Food Restriction During Gestation and Offspring Caloric Intake and Physical Activity in Middle Age: The Dutch Famine of 1944-45 Andrew Rundle; Nikolas Wada; Aryeh D. Stein; Henry S. Kahn; Karin van der Pal – de Bruin; Patricia A Zybert; L H Lumey. Dept. of Epidemiology, Columbia University, New York NY, USA; Dept. of Global Health, Emory University, Atlanta GA, USA; Division of Diabetes Translation, Centers for Disease Control and Prevention, Atlanta GA, USA; TNO Quality of Life, Leiden, Netherlands

Background: As reported elsewhere, prenatal exposure to the Dutch Famine, during which rations were <900 kcal/d from Nov 26 1944 to May 12, 1945, is associated with increased body size among adult women. The mediating roles of caloric intake and expenditure are unknown. **Methods**: We recruited two series of subjects: (1) exposed individuals, born in one of three institutions in western Holland between January 1945 and March 1946, whose mothers experienced famine during or immediately preceding pregnancy; (2) unexposed individuals, born in the same three institutions during 1943 or 1947, whose mothers did not experience famine during this pregnancy. We assessed caloric intake as derived from a 148- item food frequency questionnaire and physical activity information as derived from a 19-item questionnaire. A total activity score was derived by weighting minutes of activity per week by the intensity of the activity; the mean and standard deviation of log transformed data are reported. We defined four (partially overlapping) windows of gestational exposure (by ordinal weeks 1-10; 11-20; 21-30; and 31 through delivery) based on exposure to a western-Holland ration <900 kcal/day during the whole 10-week interval. Maternal preconception depletion was characterized by a score representing cumulative weeks of exposure to reduced rations in the 6 months prior to conception. Caloric intake and total activity score for each window of gestational exposure and for any famine exposure in gestation were compared to those of the unexposed. **Results**: For each gestational period, exposed women had higher caloric intake than unexposed women (Table) but the differences were only significant for the period 31 weeks to delivery. Physical activity did not significantly differ between unexposed and exposed, for any of the gestational periods. When exposure in any period was considered, caloric intake among men did not differ between exposed and unexposed [2437 (SD=638, N=177) vs 2384 kcal, p=0.55], nor did total activity score differ [8.12 (SD=2.91, N=177) vs 8.15, p=0.42]. Among women, caloric intake was higher among exposed women than among unexposed women [2091

kcal (SD=572, N=211) vs 1949, p=0.04], but exposure was not associated with physical activity [8.40 (SD=2.25, N=211) vs 8.55, p=0.85].

	Period of exposure to reduced rations				
Energy Intake in kcal mean, (SD), n subjects	Unexposed	Exposure wks 31-delivery	Exposure weeks 21-30	Exposure weeks 11-20	Exposure weeks 1-10
Men	2384 (702) 80	2367 (616) 65	2457 (646) 81	2546 (701) 64	2531 (660) 37
Women	1949 (496) 96	2127 (558) 77	2117 (646) 76	2089 (633) 75	2114 (519) 48
Physical Activity Scale mean, (SD), n subjects					
Men	8.15 (3.08) 80	7.88 (3.35) 65	8.02 (3.05) 81	8.19 (2.83) 64	8.19 (2.64) 37
Women	8.55 (1.71) 96	8.26 (2.62) 77	8.60 (1.92) 76	8.33 (2.63) 75	7.96 (3.20) 48

Conclusions: Among women, reduced caloric rations during gestation were associated with increased adult caloric intake. The mechanisms underlying this association remain unclear. The increase in caloric intake is consistent with increases in body size reported elsewhere.

P3-101

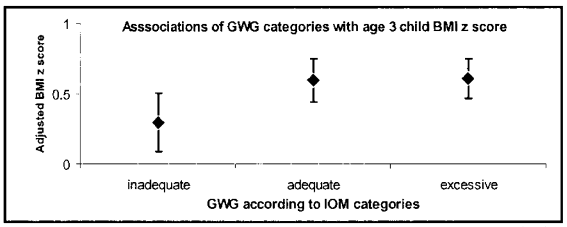
The Developmental Origins of Body Composition in Older Men and Women: Findings from the Hertfordshire Cohort Study Avan Aihie Sayer[1,2], Holly E Syddall[1], Elaine M Dennison[1], Helen J Martin[1], Sarah L Duggleby[1], David J P Barker[1], David I W Phillips[1], Cyrus Cooper[1] [1]MRC Epidemiology Resource Centre, University of Southampton [2]University Geriatric Medicine, University of Southampton

Introduction Size in early life is related to adult body mass index and it has been proposed that early environmental influences have lifelong consequences for obesity. However body mass index also reflects fat-free mass and few studies have examined the relationship between early size and more direct measures of body composition in older people. The objective of this study was to investigate the relationship between birth weight, weight at one year and body composition in older men and women. **Methods** We carried out a retrospective cohort study in Hertfordshire, UK. 1579 men and 1418 women aged 59-73 years with records of birth weight and weight at one year participated in the study. Anthropometry, including height, weight and skinfold thickness measurement, was carried out and the main outcome measures were adult body mass index (BMI), fat mass (FM) and fat-free mass (FFM). **Results** In men and women, there were significant positive relationships between birth weight and all the adult measures of body composition. However the effect sizes for BMI and FM were much smaller than for FFM. In men the correlation coefficient (95% confidence interval) for BMI was 0.07 (0.02, 0.12) p=0.004; for FM 0.07 (0.02, 0.12) p=0.004; for FFM 0.19 (0.14, 0.24) p<0.001. In women the correlation coefficient (95% confidence interval) for BMI was 0.05 (0.00, 0.10) p=0.05; for FM 0.10 (0.05, 0.15) p<0.001; for FFM 0.22 (0.17, 0.27) p<0.001. There was a similar pattern of results for weight at one year. However in men, there was also a contrast in the birth weight and weight at one year relationships with FM. The association between weight at one year and FM was stronger with a correlation coefficient 0.15 (0.10.20) p<0.001. This difference was not seen in the women. **Conclusions** The relationship between early size and adult fat-free mass was much stronger than that with fat mass or body mass index. This is consistent with previous studies demonstrating positive associations between birth weight and adult muscle mass and strength. It suggests that developmental influences have effects on muscle that persist into later life. There was some evidence that infant rather than prenatal growth was more strongly associated with adult obesity in men.

P3-102

Maternal Weight Gain During Pregnancy And Child Adiposity At Age 3 Years Emily Oken[1], Elsie M. Taveras[1], Ken P. Kleinman[1], Janet W. Rich-Edwards[1,2], Matthew W. Gillman[1,3]. [1]Department of Ambulatory Care and Prevention, Harvard Medical School and Harvard Pilgrim Health Care; Departments of [2]Epidemiology and [3]Nutrition, Harvard School of Public Health; all in Boston, MA, USA.

Background: Gestational weight gain (GWG) is directly associated with fetal growth, and higher fetal growth is associated with adiposity in childhood and adulthood. However, associations of GWG with offspring obesity have not been well studied. **Methods**: We studied 770 mother-child pairs from Project Viva, an ongoing prospective cohort study in Massachusetts, USA. Main exposures: Total GWG, defined as the difference between the last clinically recorded weight prior to delivery and the self-reported pre-pregnancy weight; and categories of inadequate, adequate, or excessive GWG according to 1990 Institute of Medicine guidelines. Main outcome: Age 3 child body mass index (BMI) z score, calculated from research-measured height and weight and standardized by child age and sex according to US national reference data. Analysis: Multivariable linear regression, adjusted for maternal prepregnancy BMI, prenatal smoking, race/ethnicity, household income, and marital status; paternal BMI; and child sex, fetal growth, and gestation length. Other maternal factors including gestational diabetes, maternal age, and other socioeconomic factors were not confounders. **Results**: Participants were 25% non-white and 94% married or cohabiting. Mean pre-pregnancy BMI was 24.7 kg/m^2 (SD 5.1) and mean GWG 15.6 kg (SD 5.5, range –7.3 to 33.2). Half (51%) of mothers had excessive GWG, and 15% had inadequate GWG. Mean age 3 BMI z score was 0.46 units (SD 1.0). GWG was directly associated with age 3 child BMI z score, with an increase of 0.07 (95% CI: 0.001, 0.14) units per 5 kg of GWG. Compared with adequate weight gain, inadequate GWG was associated with lower child BMI z score (-0.30 units, 95% CI: -0.51, -0.09) but excessive GWG was not different (0.01 units, 95% CI: -0.14, 0.16) (see Figure).



Conclusion: Maternal weight gain during pregnancy is associated with child adiposity. As childhood obesity is increasing in prevalence and effective treatment remains elusive, prevention remains critical. Maternal GWG may be a modifiable prenatal determinant of childhood adiposity.

P3-103

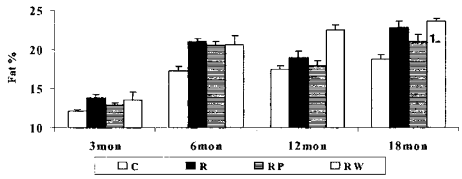
Prevalence of Obesity Among Indian Urban Slum Dwellers: A Pilot Study G.S. Toteja, Smriti Ahuja, Payal Gupa; Indian Council of Medical Research, New Delhi, India

Background: Rapid industrialization and urbanization in developing countries has contributed to the phenomenon of nutrition transition. Lifestyle changes resulting in physical inactivity and sedentary behaviour along with a shift from traditional diets to high fat/energy dense diets are responsible for the increasing epidemic of obesity. in India an overall prevalence of obesity among adults has been reported around 30% from various studies. Obesity pattern have also emerged across socio-economic groups. However, limited data is available on prevalence of obesity among low socio-economic Indians. **Sample selection:** The study has been carried out in urban slum of Delhi in India. House to house cross sectional survey was done among 170 adults. **Methodology used:** Different anthropometric measurement like height, weight were taken to compute BMI of the subjects and to assess abdominal obesity waist and hip circumference were taken and waist to hip ratio (WHR) was calculated. **Results:** The prevalence of over weight was around 19% (BMI ≥ 25 and BMI < 30) among the subjects studied. Only one female obese subject was observed (BMI ≥ 30). It was also observed that the prevalence of overweight and abdominal obesity was higher among female than males. Hence, we may conclude that in the current situation of double burden of nutrition in the developing countries, there is a need of thorough review of the prevailing situation and future trends.

P3-104

Long Term Programming of Postnatal Adiposity by Maternal and Postnatal Magnesium Status L. Venu, I. J. N. Padmavathi, Y. Durga Kishore and M. Raghunath. Endocrinology and Metabolism Division, National Institute of Nutrition, Hyderabad, India – 500 007.

Background: Prenatal under-nutrition programs the offspring to manipulated metabolic changes and adiposity in adult life. Recently we reported that maternal mineral restriction increased the body fat %, plasma lipids and oxidative stress in rat pups suggesting their predisposal to insulin resistance in later life. We have now assessed the effect of long term pre and post natal magnesium restriction / rehabilitation on the body adiposity of the offspring. **Methods:** Female weanling WNIN rats (n=21) received for 9 weeks, a 70% Mg restricted diet (R) *ad libitum* , whereas control rats (n=7) were pair fed the control AIN 93G diet , and mated with control males. Control dams and pups received control diet throughout; 7 restricted dams were shifted to control diet from parturition and their pups from weaning (RP) whereas pups of remaining Mg restricted dams received from weaning the control diet (RW) or Mg restricted diet (R). Each of the groups had 16 male pups from weaning. Body adiposity (as determined by total body electric conductivity), plasma lipid profile and adiponectin levels were analyzed in the offspring at 3, 6, 12 and 18 months of age. Parameters of oxidative stress (Malondialdehyde levels and protein carbonyls) and antioxidant status (Reduced Glutathione, catalase, superoxide dismutase and glutathione peroxidase) were monitored in the liver of the offspring on postnatal day 180. **Results:** At 3 months of age, R pups had higher body fat % and plasma triglycerides and lower lean body and fat free mass than controls and these changes were mitigated partly, in RP and RW pups at this time point. While these changes persisted in R offspring till 18 months, they were mitigated partly in only the RP but not the RW offspring (Figure). R offspring had significant insulin resistance at 6 months, which was further aggravated in RP and RW offspring. However insulin resistance did not persist in any of these three groups at the later time points tested. Although plasma adiponectin levels and tissue oxidative stress / antioxidant status were comparable among the four groups, insulin stimulated glucose uptake by muscle was lower (than controls) in R, RW but not RP offspring.



Body fat % of the WNIN rat offspring of different groups on postnatal 3, 6, 12 and 18 months as determined by TOBEC. Each bar represents a mean ± SEM (n=6) At a given age, means with different superscripts are significantly different ($p<0.05$) by one way ANOVA.

Conclusions: The persistent increase in the body fat % observed in the rat offspring till 18 months of age indicates that maternal and postnatal Mg nutritional status probably play an important role in the long term programming of body adiposity in the offspring

P3-105

Neonatal Leptin Treatment Reverses Developmental Programming in Offspring Following Maternal Undernutrition Vickers MH[1,2], Gluckman PD[1,2], Coveny AH[1,2], Hofman PL[1,2], Cutfield WS[1,2], Gertler A[3], Breier BH[1,2], Harris M[1]. [1]Liggins Institute, University of Auckland and [2]National Research Centre for Growth and Development, Auckland, New Zealand, [3]Institute of Biochemistry, Food Science and Nutrition, the Hebrew University of Jerusalem, Israel.

An adverse prenatal environment may induce long-term metabolic consequences, in particular obesity concomitant with leptin and insulin resistance. Although the mechanisms are not well understood, this "programming" has generally been considered an irreversible change in developmental trajectory. Adult offspring of rats subjected to undernutrition during pregnancy develop obesity, hyperinsulinemia and hyperleptinemia, especially in the presence of a postnatal high fat diet. Reduced locomotor activity and hyperphagia contribute to the increased fat mass. Using a well-characterised model of maternal undernutrition, the present study investigated the effects of neonatal leptin treatment on the metabolic phenotype of adult female offspring. Leptin treatment (recombinant rat leptin, 2.5 µg/g/day, sc administered as a split dose at 0800 and 1700h) from postnatal d3 to d13 resulted in a transient slowing of neonatal weight gain, particularly in programmed offspring, and normalised caloric intake, locomotor activity, body weight, fat mass, and fasting plasma glucose, insulin and leptin concentrations in programmed offspring in adult life in contrast to saline treated offspring of undernourished mothers who developed all these features on a high fat diet. Neonatal leptin had no demonstrable effects on the adult offspring of normally fed mothers. This study suggests that developmental metabolic programming is potentially reversible by an intervention late in the phase of developmental plasticity. The complete normalisation of the "programmed" phenotype in females by neonatal leptin treatment implies that leptin has effects that reverse the prenatal adaptations resulting from relative fetal undernutrition.

Oxidation; Endothelium; Vasculature

P3-106

Accelerated Maturation of CO_2-Related Brain Vasodilatory Response in Newborn aIUGR Piglets Reinhard Bauer, Bernd Walter, Thomas Rösel, Elke Gaser Institute for Pathophysiology and Pathobiochemistry, Universitätsklinikum Jena, Friedrich Schiller University, D-07740 Jena, Germany

Background: There are scant data regarding the development of cerebrovascular autoregulation in asymmetric intrauterine growth restricted (aIUGR) newborns. Recently, we have shown that aIUGR resulted in an improved ability to withstand critical periods of gradual oxygen deficit as shown by improved cerebrovascular autoregulation at hemorrhagic hypotension in an animal model of naturally occurring small-for-gestational-age term born piglet. In addition, IUGR is associated with an increased incidence of perinatal asphyxia, which results in cardiocirculatory redistribution. However, until now it is unknown whether an altered cerebrovascular regulation in aIUGR under asphyxic conditions newborns exists. **Methods**: Studies were conducted to examine the effects of intrauterine growth restriction on regulation of cerebral blood flow (CBF) and oxygen consumption ($CMRO_2$). We used 1-day old anesthetized and ventilated piglets, divided into normal weight (NW, n=40) and asymmetrical intrauterine growth restricted (aIUGR, n=39) animals. CBF was measured by colored microspheres. Brain $AVDO_2$ was determined using arterial and sagittal sinus blood samples ($CMRO_2 = CBF \eta AVDO_2$). Different stages of hypoxemia were induced for 1-h by appropriate FiO_2 lowering (moderate hypoxia: paO_2 31-34 mmHg, severe hypoxia: paO_2 20-22 mmHg). Fourteen NW and sixteen aIUGR piglets received additionally 6% CO_2 to the breathing gas, so that a $paCO_2$ of 74-80 mmHg resulted (hypoxia/hypercapnia groups). Eight NW and nine aIUGR animals served as untreated control. Results: Systemic cardiovascular and blood gas parameters, as well as hypoxia- related effects on alteration in CBF regulation and $CMRO_2$ were similar in NW and IUGR piglets. Indeed, the early period of normocapnic and hypercapnic hypoxia shows at moderate and severe O_2 deficit a marked CBF increase, maintained O_2 availability and aligned O_2 utilization resulting in maintained O_2 consumption. However, in hypoxia/hypercapnia groups cerebral oxygen extraction was markedly increased in IUGR animals ($P < 0.05$). This resulted in a significantly diminished $CMRO_2$-related increase of CBF at gradually reduced arterial oxygen content ($P < 0.05$). Furthermore, during the late period of 1-h severe O_2 deficit brain O_2 demand was reduced in NW and aIUGR piglets. $CMRO_2$ reduction amounted to about one third under normocapnic and hypercapnic hypoxia ($P < 0.05$). **Conclusions**: An enhanced effectively in oxygen availability appeared in newborn aIUGR piglets under hypoxia/hypercapnia by improved cerebral oxygen utilization. This is not connected with altered O_2 affinity of hemoglobin for oxygen or red cell oxygen transport capacity. We suggest that aIUGR newborns are more capable of protecting the brain against O_2 loss during asphyxia (hypercapnic hypoxia) than NW neonates.

P3-107

Elevated Maternal Homocysteine in Preeclampsia is Reflected in Fetal Circulation. Kristin Braekke, Anette Karlsen, Nina Kittelsen Harsem, Rune Blomhoff, Anne Cathrine Staff; Department of Pediatrics, Ulleval University Hospital, Department of Obstetrics and Gynecology, Ulleval University Hospital, Department of Nutrition, University of Oslo, Oslo, Norway

Background: Preeclampsia is characterized by pregnancy induced hypertension and proteinuria. Endothelial dysfunction is a key feature of the pathophysiology in preeclampsia. In general, endothelial dysfunction can be caused by high levels of homocysteine. Elevated maternal homocysteine concentration has been reported both in overt preeclampsia and before symptoms are present. The relationship might not be causal, but homocysteine could still have deleterious effects on the fetus. Some studies have demonstrated an inverse relationship between maternal homocysteine concentrations and fetal birth weight. The aim of this study was to explore maternal and fetal concentrations of homocysteine in pregnancies complicated by preeclampsia compared to controls, and a possible correlation between homocysteine concentrations and birth weight. **Method:** In a cross-sectional study, plasma from preeclamptic patients (n=45) and normal pregnant women (n=46) were sampled prior to delivery by cesarean section after at least 6 hours fasting. Immediately after delivery, blood from the umbilical vein and artery was obtained; all samples were kept on ice until centrifugation, and plasma was collected and frozen within one hour. Homocysteine was measured by high pressure liquid chromatography (HPLC). **Results:** Median homocysteine concentration was significantly higher in preeclamptic women compared to controls (8.2 and 6.4 µmol/L, $p<0.001$). Also in the fetal circulation we found higher levels of homocysteine in the preeclampsia group compared to controls, both in samples from umbilical vein (6,5 and 5,2 µmol/L, $p=0.005$) and artery (5.7 and 4.9 µmol/L, $p=0.006$). There was a positive correlation between maternal and fetal homocysteine concentrations (Maternal vs umbilical vein: Pearson 0,80, $p<0,001$; maternal vs umbilical artery: Pearson 0,90, $p=0,001$). Maternal homocysteine concentrations correlated negatively with baby weight percentile (Pearson -0,30, *$p= 0.009$*), but in the fetal compartments negative correlations between baby weight percentiles and fetal homocysteine concentrations were not statistically significant. **Conclusions:** Median homocysteine concentrations are higher in preeclampsia than in controls, both in maternal and fetal plasma. A role for elevated fetal homocysteine concentration could be explored, both regarding fetal growth and development of disease later in life.

P3-108

Heme Oxygenase 1 Regulates the L-Arginine/Nitric Oxide Pathway in Hypoxia in Human Umbilical Vein Endothelium Paola Casanello, Luis Sobrevia, Victoria Gallardo; Cellular & Molecular Physiology Laboratory (CMPL), Department of Obstetrics and Gynecology, Medical Research Center (CIM), School of Medicine, Pontificia Universidad Católica de Chile, PO Box 114-D, Santiago, Chile.

Background. We have previously demonstrated that hypoxia decreases L-arginine transport and nitric oxide (NO) synthesis in human umbilical vein endothelial cells (HUVEC). Similar findings were also demonstrated in fetal endothelium isolated from fetal growth restricted pregnancies (Casanello & Sobrevia, 2002). In mammalian tissues, heme-oxygenase-1 (HO-1), the inducible isoform of heme oxygenase which catalyzes the formation of carbon monoxide (CO), biliverdin and ferrous ion, is upregulated by hypoxia (Ryter & Choi, 2002). NO and CO are important physiological regulators of endothelial and vascular smooth muscle cell functions. The heme-HO and the L-arginine-NOS pathways have common characteristics and influence each others activity and function. The aim of present study was to determine whether hypoxia stimulates HO-1 induction in HUVEC and whether this induction mediates the inhibition of L-arginine transport and NO synthesis observed in hypoxia. **Methods.** HUVEC from normal term pregnancies (Ethics Committee approval and informed patient consent were obtained) were cultured in medium 199 (M199, 20% bovine sera, 3.2 mM L-glutamine), and exposed (0-24 h) to normoxia (5% O_2, ~35 mmHg PO_2 which mimics umbilical vein PO_2 *in vivo*), or hypoxia (2% O_2 ~14 mmHg PO_2) in a hypoxia chamber provided with an O_2 sensor and hypoxic gas mixture (5% CO_2, 95% N_2)(Casanello et al. 2005). L-Arginine transport (L-[^{3}H]arginine, 5-1000 µM, 2 µCi/ml, 37°C, 1 min) was measured in the presence or absence of protoporphyrin IX (ZnPP,1-100µM, heme oxygenase inhibitor). eNOS, 28S and HO-1 RNA were quantified by real-time RT-PCR. eNOS activity was determined by L-[^{3}H]citrulline formation from L-[^{3}H]arginine (4 µCi/ml, 30 min), and HO-1, β-actin, total and phosphorylated eNOS (Ser^{1177}) protein were detected by Western blot.

Results. Maximal transport velocity (V_{max}) for L-arginine transport (5.0 ± 0.3 pmol/μg protein/min) was significantly (P<0.05) reduced by hypoxia (2.8 ± 0.2 pmol/μg protein/min). In HUVEC under normoxic conditions ZnPP (1-100 □M) initially stimulated L-arginine uptake (1-3 h) while further exposure resulted in inhibition of L-arginine uptake in a time- and concentration-dependent manner (25-70%). Hypoxia decreased L-arginine uptake but no effect of ZnPP was observed. eNOS mRNA level was significantly higher at short-term hypoxia (3-6 h), but was lower at long-term hypoxia (24 h). Total eNOS protein abundance was increased by hypoxia (1.3-fold), while phosphorylated eNOS at Ser^{1177}, the active isoform of the enzyme, was reduced (92 ± 7%). Expression of HO-1 mRNA as well as the protein was enhanced by hypoxia in a time-dependent manner, paralleled with a decrease in eNOS phosphorylation at Ser^{1177} and NOS activity. **Conclusions.** Hypoxia-induced upregulation of HO-1 expression decreases L-arginine/NO pathway and reduces the catalytic activity of eNOS. These results suggest that expression of HO-1 could be involved in the inhibition of L-arginine transport induced by hypoxia in HUVEC. Supported by FONDECYT 1030607, 1030781 & 7050030 (Chile). V.G. holds a MSc-studentship from Pontificia Universidad Católica de Chile. Casanello *et al.* (2005). Circ Res. 97, 16-24. Casanello P, Sobrevia L (2002). Circ Res. 91, 127-134. Ryter SW, Choi AM (2002). Antioxid Redox Signal. 4, 625-632.

P3-109

Gestational Diabetes Increases L-Arginine Transport via a PI3-K-independent Mechanism in Human Foetal Endothelium Marcelo González, Paola Casanello, Luis Sobrevia; Cellular and Molecular Physiology Laboratory (CMPL), Department of Obstetrics and Gynaecology, Medical Research Centre (CIM), School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile.

Background: L-Arginine is the substrate for nitric oxide (NO) synthesis by endothelial NO synthase (eNOS). L-arginine transport in endothelial cells is mediated by y^+/hCAT-1 system, which is responsible for the 70-95 % of L-arginine uptake (Zharikov & Block, 1998). Human umbilical vein endothelial cells (HUVEC) obtained from patients with gestational diabetes (GD) exhibit increased L-arginine uptake (Vásquez *et al.* 2004). Insulin (0.1 nM, 8 h) increases L-arginine transport and NO synthesis in HUVEC from normal pregnancies (González *et al.* 2004). In this study we studied the role of insulin and PI3-K on L-arginine transport in HUVEC from GD. **Methods**: Human umbilical vein endothelial cells were isolated (0.2 mg/ml collagenase) from normal and GD pregnancies (Ethics Committee approval and informed patient consent were obtained) and cultured in medium 199 (M199) supplemented with 20% sera. L-[^{3}H]Arginine transport (0-1000 μM, 2 μCi/ml, 37°C, 1 min) and mRNA level (real timeRT-PCR) for hCAT-1 in presence or absence of insulin (0.1 nM, 8 h) and/or wortmannin (30 nM, 8 h, PI3-K inhibitor) was determined. **Results**: In GD cells the V_{max} of L-arginine transport was higher (1.9-fold) compared with control values in normal cells. Insulin further increased in the V_{max} for L-arginine transport (5-fold) in HUVEC from GD. The effect of insulin in GD HUVEC was partially blocked by wortmannin (~20%), however the stimulatory effect of GD on L-arginine transport was unaltered. Insulin or GD did not alter the apparent K_m for L-arginine transport (Table 1). In addition, GD increased hCAT-1 mRNA level, an effect further increased by insulin. The effect of insulin on hCAT-1 mRNA expression was blocked by wortmannin. **Conclusions**: These results suggest that the PI-3K dependent pathway may not be involved in the GD-increased L-arginine transport in HUVEC. However, the PI-3K dependent pathway is involved, at least partially, in the effect of insulin in GD HUVEC.

Source of cells (Pregnancies)	Treatment	K_m (μM)	V_{max} (pmol/μg protein/min)	V_{max}/K_m (pmol/μg protein/min/μM)
Normal	-	85 ± 24	3.9 ± 0.38	0.046
	Insulin	93 ± 20	9.9 ± 1.2	0.106
	Wortmannin	103 ± 16	4.1 ± 0.3	0.040
	Insulin+Wortmannin	98 ± 27	5.5 ± 1.1	0.056
GD	-	97.2 ± 27	7.5 ± 0.5	0.080
	Insulin	85.1 ± 25	20 ± 4	0.235
	Wortmannin	85.2 ± 26	6.9 ± 1.1	0.080
	Insulin+Wortmannin	156 ± 47	16 ± 2.7	0.103

Table 1. Kinetics parameters in HUVEC from normal and gestational diabetic pregnancies. L-[^{3}H]Arginine transport measured in presence or absence of insulin (0.1 nM, 8 h) and/or wortmannin (30 nM, 8 h). Values are mean ± S.E.M. (n=6-10). Zharikov & Block. (1998). *Biochim Biophys Acta* 1369, 173-183. Vásquez *et al.* (2004). *J Physiol* 560, 111-122. González *et al.* (2004). *Pflügers Arch-Eur J Physiol* 448, 383-394. Supported by FONDECYT 1030607, 1030781 (Chile). M.G. holds a CONICYT PhD-studentship.

P3-110

Cigarette Smoke Exposure During Pregnancy Leads to Increased Systemic Arterial Blood Pressure and Attenuated Coronary Artery Relaxation Kathleen J. Lumb, Christopher Triggle, Xiolan Zhou, Jonathan Pendlebury and Shabih U. Hasan. Department of Pediatrics and Institute of Maternal and Child Health, The University of Calgary, Calgary, Alberta, Canada

Background: Intrauterine development is a critical period for the correct patterning and organization of fetal tissues. Cigarette smoke (CS) exposure during pregnancy is the most common exogenous chemical insult to the developing fetus and may be a factor in the occurrence of adult disease including diabetes, hypertension and ischemic heart disease. **Methods and Experimental Design:** We investigated the effects of perinatal CS exposure on vascular contractile and relaxation responses, and arterial blood pressure in adult Wistar-Kyoto rats. At 100 days of age, male offspring were implanted with a femoral artery catheter attached to a radiotelemetry device for the measurement of blood pressure, heart rate and activity. This allowed us to monitor the animals from a remote location, thus avoiding erroneous readings due to handling stress. No sooner than 10 days after surgery, cardiovascular variables were recorded every 5 min for 24 hours on day 110, 120, and 130. Dose responses (log EC50) to the contractile agents 9,11-dideoxy-11a, 9a-epoxymethano-prostaglananin-F2a (U46619) for coronary artery (CA) and phenylephrine for the mesenteric artery (MA) and aorta (Ao), and the endothelium-dependent vasodilator acetylcholine (Ach) were obtained. Subsequently, the effects of indomethacin, L-NAME, the soluble guanylyl cyclase inhibitor (ODQ), apamin, charybdotoxin and K^+ on the Ach mediated vascular relaxation were investigated. Random effects generalized least square regression analyses were performed to investigate the independent effects of age and treatment on systemic arterial blood pressures and heart rate. **Results:** Our data suggest that as compared with the control group, the CA of CS exposed animals demonstrated a decreased relaxation response to Ach and that addition of L-NAME completely inhibited the Ach mediated relaxation. The arterial blood pressure, measured using radiotelemetry devices, was also significantly higher in CS exposed group. Furthermore, the changes in vascular responsiveness were independent of nutritional status of the animals. **Conclusions:** We present novel and exciting evidence that prenatal CS exposure has long-term deleterious effects on vascular endothelial and/or smooth muscle integrity. It is likely that CS exerts such aberrant vascular changes during fetal angiogenesis. Our data are not only important for raising public awareness but may also provide a basis for future gene targeting and therapeutic interventions in humans.

P3-111

Oxidant Stress in LPS-Induced Preterm Birth in Mice Kathryn M. Heyob, Floor A. Jenniskens, Lynette K. Rogers, Stephen E. Welty, Charles V. Smith Columbus Children's Research Institute, Department of Pediatrics, Ohio State University, Columbus, OH

Background: Preterm delivery is the leading cause of morbidity and mortality in neonates. Sub-clinical maternal infections caused by gram-negative bacteria are thought to be a major cause of preterm labor. In a recent report [AJOG 2003; 188: 203-8], premature delivery and fetal death were observed in C57BL/6 mice administered 10 μg of LPS at E16. Decreases in maternal and fetal levels of glutathione (GSH) were observed, and oxidant mechanisms were proposed, but no data on biomarkers of oxidant activities were reported. **Objective**: The present studies were designed to test the hypothesis that oxidant stresses, as evidenced by increased levels of glutathione disulfide (GSSG) in the liver and lung, occur in conjunction with LPS-induced fetal demise and preterm delivery in mice. **Methods**: C57BL/6, glutathione-reductase (GR) deficient GR1a1Neu, their control C3H/HeN, and Tlr-4 mutant C3H/HeJ mice were injected i.p. with highly purified LPS (*E. coli* 0111:B4; 10 μg/mouse) or sterile saline on day 16 of pregnancy (E16). The animals were followed to parturition or were sacrificed at 2 or 6 h after injection. Livers and lungs were taken from the dams, and livers taken from the fetuses. One to three fetuses/litter were formalin-fixed whole for subsequent histological analyses. Tissue GSH and GSSG levels and GR activities were measured using enzyme-recycling methods. Results are reported as means ± SE. Data were assessed by 2-way ANOVA, with modified t-tests post hoc, and differences attributed at p<0.05. **Results**: Through 6 h after LPS injection, the dams of all strains appeared normal. LPS-treated C57BL/6, C3H/HeN, and Neu dams delivered between 14 and 16 h post dose, whereas LPS-treated C3H/HeJ mice carried to term with delivery of live pups. No differences in lung GSH or GSSG levels were observed among dams of any strain. In C57BL/6 dams, hepatic GSH levels were lower 2 and 6 h after LPS treatment (7.66 ± 1.17 and 5.35 ± 1.13 μmol/g liver, respectively) than in the saline-treated animals (10.29 ± 1.48 and 7.65 ± 0.47), and the levels were lower 6 h after LPS than after 2 h (p<0.01). No changes were observed in hepatic GSSG levels in dams. In the fetuses, however, GSSG levels 6 h after LPS (20.74 ± 8.88 nmol/g liver) were higher than 2 or 6 h after saline (7.53 ± 2.68 and 10.09 ± 8.28) and 2 h after LPS (7.32 ± 2.92 nmol/g) (p<0.001). Fetal hepatic GSH levels 6 h after LPS (1.77 ± 0.23 μmol/g liver) were lower than in either saline-treated group (2.13 ± 0.64) or 2 h after LPS (2.33 ± 0.28) (p<0.01). Two hours after LPS dosing, livers of Gr1a1Neu dams had higher GSSG levels (97.84 ± 10.44 nmol/g) than 6 h after LPS and in the C3H/HeN controls (58.69 ± 7.89 and 49.71 ± 8.54 nmol/g). Fetal liver GSSG levels were higher in both Neu and HeN mice 6 h after LPS treatment (80.99 ± 4.92 and 54.55 ± 3.49 nmol/g) than in saline-treated mice (35.26 ± 4.92 and 20.57 ± 3.55 nmol/g). Fetuses from LPS-treated C3H/HeN dams had lower hepatic GR activities at 6 h than did fetuses from saline-treated dams (4.59 ± 2.08 vs. 13.54 ± 1.88 mU/mg protein). **Conclusion**: The decreases in hepatic GSH levels in dams support a rationale for therapeutic interventions with N-acetylcysteine or other GSH precursors. The increases in hepatic GSSG levels in fetuses observed 6 h after maternal LPS suggest other opportunities for therapeutic interventions, such as in oxidant-mediated activation of signal transduction pathways.

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Increased Fetal Vascular Resistance in Gestational Diabetes Mellitus

Terence T. Lao, Ghazala S. Basir, Wing-Cheong Leung, Helena S.W. Lam, Pik-To Cheung*; Departments of Obstetrics & Gynaecology and Paediatrics*, The University of Hong Kong; Queen Mary Hospital, Pokfulam Road, Hong Kong SAR, PRC.

Background: To determine the effect of gestational diabetes mellitus (GDM) on fetal placental and cerebral vascular resistance indices at term and the relationship between these indices with glycaemic control and maternal and fetal characteristics. **Methods**: In a prospective study, 109 women with GDM and 90 non-diabetic controls were recruited from the high-risk antenatal clinic at 35-37 weeks gestation for Doppler studies of the fetal umbilical (UA) and middle cerebral (MCA) arteries. The UA signals were obtained from a free-floating section of the umbilical cord. The MCA signals were obtained at its origin from the Circle of Willis on a transverse section of the fetal head at the level of the cerebral peduncles using color Doppler. The sample gate, set at the lowest high-pass filter and the smallest sample volume, was applied to the medical third of the proximal portion of the MCA. Doppler blood flow indices were recorded from an angle of insonation close to 0° with minimal pressure to avoid compression of fetal head. One investigator performed all the scans and three consecutive waveforms of similar configuration from each vessel were obtained. The mean values of the systolic diastolic ratio (S/D), resistance index (RI) and pulsatility index (PI) were calculated by the machine and compared between the GDM and control groups, as well as correlated with maternal and fetal characteristics in the entire cohort and with parameters of glycaemic control in the GDM group. **Results**: The GDM group had significantly increased maternal age, weight, and body mass index, and shorter gestational age ($p<0.01$ for all), but there was no difference in maternal or fetal weight at Doppler examination, and the mean birthweight was similar. The GDM group had increased S/D (3.57±0.77 vs 3.19±0.63), PI (1.29±0.25 vs 1.17±0.22), and RI (0.72±0.12 vs 0.67±0.07) for the MCA ($p=0.001$), and increased RI (0.57±0.12 vs 0.53±0.07, $p=0.049$) for the UA. For the entire cohort, inverse correlation ($p<0.05$) was found between fetal femur length with UA S/D (rho=-0.146), UA RI (rho=-0.143), and abdominal circumference with UA S/D (rho=-0.173). In the GDM group, fetal femur length was inversely correlated ($p<0.05$) with UA S/D (rho=-0.213) and UA RI (rho=-0.212), while all the UA indices were positively correlated ($p<0.05$) with maternal fructosamine level (for S/D rho=0.223, for PI rho=0.223, for RI rho=0.313). For the MCA, only the PI was inversely correlated ($p<0.02$) with mean glucose level in the week of the scan (rho=-0.258). **Conclusions**: Maternal GDM is associated with increased fetal cerebral, but not placental, vascular resistance at term. The UA S/D and RI were inversely correlated with femur length, suggesting that placental vascular resistance influences fetal growth in terms of length/height. Short term maternal glycaemic control, as reflected in the fructosamine level, affects the placental vascular resistance, while the MCA vascular resistance is affected by the most recent glycaemic control. Further studies are necessary to determine whether the increased fetal cerebral vascular resistance will persist after birth.

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Endothelial Mechanisms Regulating Uteroplacental Blood Flow and Fetal Growth during Gestation

Ronald R. Magness[1,2], Dong-bao Chen[3], Ian M. Bird [1], and Jing Zheng [1] Perinatal Research Labs, Departments of Obstetrics & Gynecology[1] and Animal Sciences[2], University of Wisconsin-Madison, Madison WI; Department of Reproductive Medicine, University of California San Diego, Lo Jolla, CA[3]

During normal gestation uterine and placental blood flows increase dramatically in order to support adequate fetal growth. Blood flows at the maternal-fetal interface develop from both vasodilatation and angiogenesis. Understanding of these mechanisms is important because insufficient uterine/placental blood flows lead IUGR resulting in increased fetal morbidity and long-term developmental origins of adult onset diseases (i.e. the Barker Hypothesis). Endothelial nitric oxide (NO) synthase (eNOS) expression and NO/cGMP production are elevated in the uterine and placental endothelial cells during the third trimester of ovine pregnancy when blood flows and fetal growth are increasing. Inhibition of NO production reduces blood flows in both the maternal and fetal compartments. Locally produced and systemic endocrine factors, including the elevated angiogenic factors, estrogen and fluid shear stress, interact to regulate NO production and thus reproductive blood flows in normal pregnancy. Placental angiogenic factors and NO play vital roles in regulating both placental angiogenesis as well as vasodilatation. During the third trimester of pregnancy paralleling fetal growth and uterine and placental blood flows, we observed elevations in fetal placental angiogenic activity, bFGF and VEGF secretion, and NO production. Both bFGF and VEGF have the capability of increasing uterine and placental endothelial NO production via activating eNOS and/or increasing eNOS expression. Placenta-derived estrogen is a potent uterine vasodilator and its levels are also elevated in normal gestation. Blockade of estrogen receptors (ERs) using ICI 182,780 lowers gravid UBF the same amount as the fall seen with NO inhibition using L-NAME. Estrogen also increases *de novo* NO production by uterine artery endothelial cells (UAECs) via an ER and ERK1/2 mediated mechanism. Increases in UBF elevate laminar/pulsatile shear stress and uterine vascular NO production. Unlike static UAEC cultures, in the presence of basal shear stress, E2β dramatically augments the rise in eNOS protein expression. Thus, regulation of coordinated rises in blood flows at the maternal-fetal interface is modulated by convergent endothelial NO associated mechanisms that are important to fetal development.

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Vascular Dysfunction in the Offspring of Protein Restricted Pregnant Rat Dams Can Not be Explained by Changes in eNOS, iNOS or sGC Gene Expression

Joanne Rodford, Christopher Torrens, Timothy Wheeler, Mark A. Hanson and Geraldine F. Clough; Centre for Developmental Origins of Health and Disease, University of Southampton, Southampton SO16 5YA, UK

Background In the rat, dietary protein restriction during gestation results in growth restriction of the offspring, along with hypertension and vascular dysfunction. Previous studies have suggested an alteration in the NO/sGC pathway in the vessels of these animals (Brawley *et al.*, 2003; *Ped Res.* **54**, 83-90), however, few studies have investigated molecular changes in this pathway in the protein-restricted offspring. This study investigated alterations in gene expression of key enzymes involved in the relaxation of resistance arteries in male offspring of protein restricted dams. **Methods** Two groups of female Wistar rats were fed diets of either 18% protein (control: C, n = 6) or 9% protein (protein-restricted: PR, n = 7) throughout gestation. The offspring were weighed at birth and weekly up to 120 days of age. Male offspring were culled and small mesenteric arteries (~ 250 μm diameter) dissected, mounted in a wire myograph in physiological salt solution, heated to 37 °C and gassed with 95% O_2 and 5% CO_2. Following normalisation, functional integrity was assessed with constriction to 125 mM KPSS. Cumulative response curves were constructed to phenylephrine (PE; 10 nM – 100 μM), angiotensin II (Ang II; 10 pM – 100 nM) and acetylcholine (ACh; 1 nM – 10 μM). RNA was extracted from similar mesenteric arteries and mRNA levels of endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS) and soluble guanylate cyclase (sGC) measured using TaqMan quantitative real-time PCR and normalised using 18S RNA. Data are presented as mean ± SEM and differences assessed by two-way repeated measures or two-way analysis of variance (ANOVA). Significance was accepted at $p<0.05$. **Results** While there was no difference in mean birthweight of the offspring, there was a significant reduction in growth rate of the PR vs. C males to 120 days ($p<0.01$). Vasoconstriction to PE and Ang II was not different between the groups. ACh-induced vasodilatation was significantly blunted in PR vs. C arteries (pEC_{50}: C, 8.02 ± 0.06, n=10; PR, 7.55 ± 0.14, n=8; $p<0.05$). However, levels of eNOS, iNOS and sGC mRNA were not different between the two groups. **Conclusions** The lack of difference in vasoconstriction between the two groups suggests that there is no change in smooth muscle function in the mesenteric arteries. The lack of alteration in eNOS, iNOS or sGC mRNA expression in association with the blunted ACh-induced vasodilatation suggests that the vascular dysfunction is not due to gene regulation of these enzymes, but perhaps to post-transcriptional modifications, enzyme activity or the bioavailability of substrates and cofactors. This study was supported by the British Heart Foundation

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Equilibrative Nucleoside Transporter 1 is Downregulated by Activation of A_{2a} Adenosine Receptors in Human Umbilical Vein Endothelium.

Rody San Martín, Francisco Minaya, Marcelo Farías, Paola Casanello, Luis Sobrevia; Cellular and Molecular Physiology Laboratory (CMPL), Obstetrics and Gynaecology Department, Medical Research Centre (CIM), School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile.

Background. High D-glucose and gestational diabetes increase nitric oxide (NO) synthesis and reduce nucleoside transport in human umbilical vein endothelium (HUVEC)(see review, San Martín & Sobrevia, 2005). HUVEC from gestational diabetes also exhibits increased extracellular levels of adenosine ending in adenosine receptor activation (Vásquez et al. 2004). We studied the involvement of adenosine and A_{2a} purinergic receptor in the expression and activity of the equilibrative nucleoside transporter 1 (hENT1) known to be the major membrane transport proteins mediating adenosine uptake in HUVEC. **Methods.** Adenosine transport (7.5-500 μM, 4 μCi/ml, 37°C, 20 s) was measured in passage 2 cells in medium 199 (3.2 mM L-glutamine, Ethic committee approval and informed patient consent obtained) in presence of CGS-21680 (30 nM, A_{2a} agonist) or ZM-241385 (10 nM, A_{2a} antagonist) and hypoxanthine (2 mM, hENT2 inhibitor). hENT1 protein content in HUVEC was determined by Western blot. hENT1 gene promoter activity was measured in HUVEC exposed to adenosine (10 μM, 4 h) following transfection by electroporation with pGL3 (luciferase reporter gene) plasmids carrying -3100, -2056 and -1016 bp of the promoter sequence (320 Volt, 30 ms, 8-10% efficiency). **Results.** hENT1-mediated adenosine uptake is reduced in HUVEC exposed to CGS-21680 (V_{max} = 1.5 ± 0.3 vs 10.2 ± 5.6 pmol/μg protein/min, n=6). The inhibitory effect of CGS-21680 is blocked by the A_{2a} antagonist ZM-241385. hENT1 protein content is reduced by ~50% in HUVEC exposed to adenosine 10 μM. Transcriptional activity of hENT1 promoter sequence from -2050 up to -3100 bp is decreased by adenosine 10 μM, while transcriptional activity of the proximal sequences (up to -1016 bp) was unaltered by this nucleoside. **Conclusion.** hENT1 is a target for A_{2a} adenosine receptors signalling, down-regulating nucleoside transport activity in HUVEC which could explain the increased extracellular adenosine levels detected in HUVEC from gestational diabetes. In addition, hENT1 expression is repressed at transcriptional and post-translational level by adenosine in HUVEC. Vázquez et al. (2004). J Physiol 560, 111-122. San Martín R, Sobrevia L (2005). (doi:10.1016/j.placenta.2005.01.011)

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Insulin Restores the Elevated Adenosine Transport in Human Umbilical Vein Endothelial Cells from Intrauterine Growth Restriction Pregnancies

Felipe Sanhueza, Paola Casanello, Luis Sobrevia; Cellular and Molecular Physiology Laboratory (CMPL), Department of Obstetrics & Gynaecology, Medical Research Centre (CIM), School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile.

Background. Intrauterine growth restriction (IUGR) is a perinatal disease associated with intrauterine hypoxia, diminished fetal growth, decreased plasma insulin levels and vascular dysfunction in the adulthood. The endogenous nucleoside adenosine mediates vasodilatation via activation of A_{2a} purinoceptors and L-arginine/nitric oxide (NO) pathway in human umbilical vein endothelial cells (HUVEC). Extracellular adenosine levels are regulated by the activity of equilibrative nucleoside transporters (hENTs) 1 and hENT2 in HUVECs (Aguayo et al. 2005) and play crucial roles in hypoxia (Casanello et al. 2005) and gestational diabetes (San Martin & Sobrevia, 2005) where level of insulin may be altered. The present study aims to determine whether insulin restores adenosine transport in IUGR HUVEC. **Methods.** HUVEC from normal or IUGR pregnancies (Ethics Committee approval and informed patient consent were obtained) were cultured in medium 199 (M199, 20% bovine sera, 3.2 mM L-glutamine). Confluent cells were exposed to 1 nM insulin (1 nM) for 8 h. Adenosine transport kinetics (7.8 to 500 μmol/L) was measured in absence or presence of 1 μM nitrobenzylthioinosine (NBMPR, 30 min, inhibitory concentration for hENT1) or 10 μM NBMPR (inhibitory concentration for hENT1 and hENT2). hENT1 mRNA levels was quantified by real-time RT-PCR. hENT1 and hENT2 protein content was detected by Western Blot. **Results.** Overall adenosine transport is higher in IUGR compared to normal HUVECs (0.28 ± 0.03 vs 0.12 ± 0.01 pmol/μg protein/min, respectively). There is no difference in hENT1-mediated adenosine transport between normal and IUGR HUVECs. hENT2-mediated adenosine transport in IUGR is significantly higher than the basal values observed in normal HUVEC (V_{max} = 0.12 ± 0.01 vs 0.02 ± 0.001 pmol/μg protein/min, respectively). NBMPR inhibits adenosine transport in normal and IUGR HUVECs, in a dose-response manner with similar apparent K_i values of hENT1 (K_i = 0.49 ± 0.01 vs 0.92 ± 0.01 nM, respectively). Adenosine transport in normal and IUGR HUVEC was sodium-independent. In normal HUVEC insulin increased adenosine transport via mediated by hENT2 (V_{max} = 2.87 ± 0.02 vs 0.92 ± 0.03 pmol/μg protein/min for normal with insulin vs control, respectively) and decreases transport mediated by hENT1 (V_{max} = 2.73 ± 0.02 vs 3.03 ± 0.04 pmol/μg protein/min for insulin vs control, respectively). However, while in IUGR HUVEC insulin is able of reverse the IUGR-associated increase of hENT2-mediated transport (V_{max} = 0.82 ± 0.02 vs 2.65 ± 0.05 pmol/μg protein/min for insulin vs control, respectively), and recovered the hENT1-mediated transport to normal values (V_{max} = 1.23 ± 0.03 vs 1.25 ± 0.03 pmol/μg protein/min for insulin vs control, respectively). hENT1 mRNA and protein were reduced in IUGR and hENT2 protein content was is increased in IUGR compared with to normal HUVEC. **Conclusions.** These results show that IUGR HUVECs have an increased adenosine transport mediated by hENT2, an effect that is reverted in the presence of insulin suggesting a crucial role for this hormone in the modulation of adenosine transport in human foetal endothelium. Casanello et al. (2005). Circ Res. 97, 16-24. San Martín R, Sobrevia L (2005). Placenta (doi:10.1016/j.placenta.2005.01.011) Sobrevia et al (1997). J Physiol 498, 787-796. Ong et al. (2000). J Clin Endocrinol Metab 85, 4266-4269. Aguayo et al. (2005). Placenta (doi:10.1016/j.placenta.2004.10.006) Supported by FONDECYT 1030607, 1030781 & 7050030 (Chile).

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Differential Effects of Pre- & Peri-conceptional Nutrient Restriction On Renal Vascular Reactivity in Adult Sheep Offspring

M. Shanmuganathan[1], C. Torrens[1], D.E. Noakes[2], L. Poston[3], M.A. Hanson[1] & L.R. Green[1]. [1]Centre for Developmental Origins of Health & Disease, University of Southampton, SO16 5YA. [2]Department of Veterinary Reproduction, Royal Veterinary College, AL9 7TA [3]Division of Reproductive Health, Endocrinology & Development, GKT Hospitals, London, SE1 7EH.

Background There is now considerable interest in the effects of maternal environment in very early pregnancy on offspring cardiovascular health. Early gestational maternal nutrient restriction is associated with altered cardiovascular control in adult sheep offspring and renal mechanisms are implicated (Cleal et al., 2004, Journal of Physiology 565P C156; Gardner et al., 2004, Hypertension 43, 1290-1296). We investigated the effect of pre- and peri-conceptional nutritional estriction on renal artery reactivity in female adult sheep offspring. **Methods** Welsh Mountain ewes (UK Animals (Scientific Procedures) Act, 1986) were fed 100% nutrient requirements (control, C, n=8), 50% total nutrient requirements for 30 days prior to conception (pre-conception, PRE, n=12) or 50% total nutrient requirements for 15 days before and 15 days after conception (peri-conceptional, PERI, n=12) and 100% thereafter. At 3.5 years of age female offspring were euthanized with sodium pentobarbitone (0.8 ml/kg i.v.) and the right major renal artery dissected. The renal artery was cut into 2 mm segments and mounted in an organ bath. Reactivity was assessed with cumulative concentration-response curves to phenylephrine (PE), angiotensin II (Ang II), acetylcholine (ACh), isoprenaline, forskolin and sodium nitroprusside (SNP). Data are expressed as mean ± S.E., differences were analysed by one-way analysis of variance with Bonferroni post-hoc correction for multiple comparisons. Significance was accepted if $p<0.05$. **Results** All arteries produced a pronounced vasoconstriction to PE, which was blunted in the PRE group compared to the PERI group (pEC_{50}: C: 5.94 ± 0.07, n=8; PRE: 5.76 ± 0.03, n=12; PERI: 5.93 ± 0.05, n=12; $p<0.05$). Vasoconstriction to Ang II was considerably less than that to PE and was not different between the groups. Both ACh and isoprenaline failed to produced any discernable dilatation. Forskolin produced a concentration-dependent vasodilatation, although this was not different between the groups. Vasodilatation to SNP was however significantly impaired in the PERI group compared to controls (pEC_{50}: C: 5.72 ± 0.11 n=8; PRE: 5.87 ± 0.12 n=12; PERI: 5.27 ± 0.07, n= 12; $p<0.01$). **Conclusions** This study demonstrates altered adult renal artery reactivity following a pre-conceptional and peri-conceptional dietary challenge. Sympathetic vasoconstrictor and smooth muscle vasodilator mechanisms are differentially affected by the timing of the insult and may have implications for the development of cardiovascular disease in later life. This work was supported by the British Heart Foundation

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Impaired Femoral Resistance Artery Function in Adult Sheep Offspring Following Pre- or Peri-conceptional Nutrient Restriction

C. Torrens[1], D.E. Noakes[2], L. Poston[3], M.A. Hanson[1] & L.R. Green[1]. [1]Centre for Developmental Origins of Health & Disease, University of Southampton, SO16 5YA. [2]Department of Veterinary Reproduction, Royal Veterinary College, UK, AL9 7TA [3]Division of Reproductive Health, Endocrinology & Development, GKT Hospitals, London, SE1 7EH.

Background There is now considerable interest in the effects of maternal environment in very early pregnancy on offspring cardiovascular health. Unbalanced nutrition throughout pregnancy produces effects on the offspring vascular function (Brawley et al., 2003, Pediatric Research **54**, 83-90) however, it is not known whether similar effects can be induced in early pregnancy alone. We investigated the effects of pre- and peri-conceptional nutrient restriction on responses in isolated small femoral arteries of female adult sheep offspring. **Methods** Welsh Mountain ewes (UK Animals (Scientific Procedures) Act, 1986) were fed 100% nutrient requirements (control, C, n=8), 50% total nutrient requirements for 30 days prior to conception (pre-conceptional, PRE, n=12) or 50% total nutrient requirement for 15 days before and 15 days after conception (peri-conceptional, PERI, n=12) and 100% thereafter. At 3.5 years of age female offspring were euthanized with sodium pentabarbitone (0.8 ml/kg i.v.) and the femoral artery dissected. Small branches (ca. 300 μm) were and mounted in a wire myograph. Reactivity was assessed with cumulative concentration-response curves to phenylephrine (PE), thromboxane (U46619), acetylcholine (ACh), bradykinin (BK) and sodium nitroprusside (SNP). Data are expressed as mean ± S.E. and differences were analysed by one-way analysis of variance with Bonferroni post-hoc correction for multiple comparisons. Significance was accepted if $p<0.05$. **Results** No changes in birth weight, growth, body composition or weight at time of study were noted between the three groups. Vasoconstriction to PE and U46619 was similar in all three groups. Endothelial-dependent vasodilatation to ACh was blunted in both the PERI and the PRE groups compared to the controls (pEC_{50}: C, 8.61 ± 0.07, n=6; PRE, 8.32 ± 0.05, n=11; PERI, 7.96 ± 0.10, n=7; $p<0.01$, C vs. PRE vs. PERI), whilst vasodilatation to BK was similar in all groups. In addition, vasodilatation to SNP was blunted in the PERI group compared to controls (pEC_{50}: C, 9.19 ± 0.18, n=5; PRE, 8.20 ± 0.17, n=9; PERI, 7.27 ± 0.50, n=6; $p<0.01$, C vs. PERI). **Conclusions** These data suggest that subtle changes in maternal nutrient environment, not only during the preimplantation stage but also prior to conception, influence cardiovascular function in the adult offspring. As with other models of maternal undernutrition the effect is on the NO/cGMP pathway of vasodilatation. This work was supported by the British Heart Foundation

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Effect of Transforming Growth Factor ß1 on L-Arginine/Nitric Oxide Pathway in Human Umbilical Vein Endothelium Exposed to High D-Glucose

Rodrigo Vásquez, Paola Casanello, Luis Sobrevia; Cellular & Molecular Physiology Laboratory (CMPL), Department of Obstetrics and Gynaecology, Medical Research Center (CIM), School of Medicine, Pontificia Universidad Católica de Chile, P.O. Box 114-D, Santiago, Chile.

Background: High D-glucose concentration increases L-arginine transport and nitric oxide (NO) synthesis in human umbilical vein endothelial cells (HUVEC)(Sobrevia *et al.* 1996) by unknown mechanisms. Diabetes mellitus and hyperglycemia are associated with increased synthesis and release of Transforming Growth Factor Beta 1 (TGF-ß1)(McGinn *et al.* 2003; Flores *et al.* 2004). However, the effects of TGF-ß1 on L-arginine/NO pathway have not yet been clearly understood in endothelial cells. The purpose of this study was to determine the role of TGF-ß1 on D-glucose stimulated L-arginine/NO pathway. **Methods:** HUVEC from normal pregnancies (Ethics Committee approval and informed patient consent were obtained) were cultured in medium 199 (M199) containing 20% sera, 3.2 mM L-glutamine, 100 IU/ml penicillin-streptomycin (37 °C, 5% CO_2). Confluent cells were cultured for 2 h in M199 with 2% serum, and then exposed (0-24 h) to 5 mM D-glucose, 5 mM D-glucose + TGF-ß1 (0.01-10 ng/ml), 25 mM D-glucose, or 25 mM D-glucose + TGF-ß1. L-[^{3}H]Arginine transport (15-1000 μM, 2 μCi/ml, 37°C,

1 min) was determined. Human Cationic Amino acid Transporter 1 (hCAT-1) mRNA was quantified by real time RT-PCR using 28S rRNA as housekeeping gene. NO synthase (NOS) activity was determined by L-citrulline assay, incubating cells with L-[^{3}H]arginine (4 μCi/ml, 37°C, for the last 30 min of the incubation period), in absence or presence of L-NAME (100 μM). Total and active TGF-ß1 level in the supernatant was measured by ELISA. Phosphorylated (Ser^{1177}-P~eNOS) and total endothelial NOS (eNOS), Smad2 and $p42/44^{mapk}$ protein levels were determined by Western blot. **Results:** L-Arginine transport is increased by TGF-ß1 at 1-6 h of incubation with a half maximal concentration (K_s) at 6 h of 1.92 ± 0.3 ng/ml. High D-glucose (25 mM) stimulates L-arginine transport between 1 to 24 h incubation. L-Arginine transport did not further increase when cells were co-incubated with 25 mM D-glucose and different TGF-ß1 concentrations. L-Arginine transport increase was correlated with a higher V_{max}, without changes in the apparent K_m for L-arginine transport, and hCAT-1 mRNA expression. Incubation with 25 mM D-glucose increased active and total TGF-ß1 release from HUVEC at 6 h, but decreased at 24 h. Phosphorylated Smad2 content, an indicator of active TGF-ß1 signaling, increased with D-glucose and TGF-ß1 during all the incubation time. High D-glucose and TGF-ß1 increases phosphorylated eNOS and total eNOS protein levels, and L-citrulline formation at 6 h and 24 h. Phosphorylated $p42/44^{mapk}$ content was increased by D-glucose at 6 h and 24 h, and by TGF-ß1 only at 6 h. **Conclusions:** High-D-glucose-stimulated L-arginine/NO pathway could be explained by a mechanism involving TGF-ß1-dependent stimulation of L-arginine transport during the first 6 h, involving activation of $p42/44^{mapk}$. The long-term effect of D-glucose (24 h) on L-arginine transport seems TGF-ß1 independent. However, long-term enhanced eNOS activity by D-glucose could be dependent on the activation of the intracellular TGF-ß1-mediated downstream Smad2 signaling molecule. Supported by FONDECYT 1030607 & 1030781 (Chile). R.V. holds a DIPUC-School of Medicine PhD fellowship. Sobrevia *et al.* (1996). *J Physiol* 490, 775-781. McGinn *et al.* (2003). *Am J Physiol* 284, C1374-1386. Flores *et al.* (2004). *Diabet Med* 21, 285-289.

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