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MEETING ABSTRACTS

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PLACENTAL EPIGENETIC ALTERATIONS AND VITAMIN D SIGNALING IN PREECLAMPSIA

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Objectives: Epidemiologic data implicate maternal hypovitaminosis D in the pathogenesis of preeclampsia (PE). Hypovitaminosis D prevalence is increasing globally and is a particular problem among individuals at northern latitudes. In order to elucidate the regulation of vitamin D signaling in preeclampsia, we determined DNA methylation patterns and protein expression in genes central to vitamin D metabolism in human placentas from women with normotensive pregnancy (NP) and pregnancy complicated by PE. **Methods:** Genome-wide DNA methylation patterns were determined in placental tissue from women with NP (control) and PE (n=3/group), Samples were labeled and co-hybridized to Human DNA Methylation 2.1M microarrays (NimbleGen). Raw data were analyzed using NimbleScan software producing signal ratio values and estimating the significance of enrichment. Promoters and genes with DNA methylation differences (hypo or hypermethylated) in experimental vs control samples were identified. Criteria for gene selection included methylation within 1000 bp upstream or downstream of the respective gene's transcription start site. We identified 163 genes which were uploaded to DAVID (http://david.abcc.ncifcrf.gov), providing functional interpretation of genes. In this study, we targeted genes producing proteins involved in vitamin D signaling. Western blot analysis was used to determine protein expression in differentially methylated genes. **Results:** The gene encoding 1α-hydroxylate was not differentially methylated, though protein expression was significantly increased in PE vs. NP samples ($p<0.05$). The promoter region for the vitamin D receptor (VDR, gene id 7421) which encodes the nuclear hormone receptor for vitamin D3 was methylated near the transcription start site in placentas from women with preeclampsia, suggesting that expression of this gene is downregulated in preeclampsia. Protein expression of the VDR was reduced in PE, but did not reach the level of significance. Retinoid X receptor alpha (RXRα, gene id 6256) which is a significant contributor to VDR mediated gene expression was also found to be methylated in the promoter region in placentas from women with preeclampsia compared to those unmethylated in normal pregnancy. Protein expression of RXRα was significantly reduced in PE compared to NP ($p<0.01$). **Implications:** Vitamin D insufficiency in pregnancy is associated with increased blood pressure and risk for preeclampsia, leading to increased cardiovascular risk in mothers and children. Placental DNA methylation in vitamin D receptor complex genes may reduce vitamin D responsive gene transcription thereby reducing utilization in the placenta. As DNA methylation patterns are heritable, such changes may be involved in programming of the fetal cardiovascular system for future risk of preeclampsia and hypertension.

GENES ASSOCIATED WITH IMMUNE RESPONSE AND RISK OF PRE-ECLAMPSIA IN AN AMERICAN INDIAN POPULATION

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Objectives: The etiology of pre-eclampsia (PE) is unknown; but maladaptive maternal immune responses have been hypothesized as pathogenic factors. Since population specific prevalences of variants and background genetic effects may influence risk in different communities, our aims were to: 1) determine the prevalence of 3 single nucleotide polymorphisms (SNPs) of three genes affecting immune function within this American Indian community; and 2) to investigate possible association of these variants with PE. **Methods:** A case-control study has enrolled cases confirmed by chart review and controls, matched on date of the index infant's birth. Genotyping utilized commercially available, allele specific, real-time PCR assays. **Results:** Genotypes have been obtained for 104 cases and 200 controls. Hardy-Weinberg equilibrium is satisfied for each of the 3 polymorphisms. The estimated population allele frequency of the risk allele (G) is 30% among controls. Conditional logistic regression analysis of the non-synonymous rs231775 SNP of the cytotoxic T lymphocyte associated gene (*CTLA4*) shows an increased odds ratio of 1.25 ($p=0.181$, CI 0.90 - 1.74) for each additional G allele in unadjusted analysis. In a multivariate model including maternal age, nulliparity, BMI (all independently significant with $p<0.05$), the odds ratio is increased to 1.52 ($p=0.038$, CI 1.02 – 2.27). The *MBL2* SNP (rs1800451) is marginally associated with PE in a T allele dominant, multivariate model, with an odds ratio of 3.17 ($p=0.058$, CI 0.96-10.44). The *IL1A* SNP (rs 3783550) showed no evidence of association in various models. **Conclusions:** This *CTLA4* variant has been associated with pre-eclampsia in two other disparate populations (Finnish and Iranian) and is known to be expressed in decidual tissue (1,2,3). Since this test of *CTLA4* corroborates earlier findings, the Bonferroni correction may be overly conservative, thus these data further support the role of genetic influences on immune function and resultant risk for pre-eclampsia. 1 Samsami Dehaghani A et al. (2005) Int J Gynaecol Obstet. 88(1):19-24. 2 Jaaskelainen E et al. (2008) Clin Chem Lab Med. 46:169–173. 3 Wang X et al. (2006) Int J Gynaecol Obstet. 93:123–9.

THE EFFECT OF MATERNAL NUTRITIONAL PLANE DURING PREGNANCY ON POSTNATAL BONE DEVELOPMENT IN OFFSPRING IN SHEEP

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Objectives: The objectives were to determine the effects of maternal nutritional plane during gestation on bone development and mineralization processes of the offspring during early postnatal development. **Methods:** 84 Rambouillet ewes (age = 240 ± 17 d, BW = 52.1 ± 6.2 kg) were allocated to one of three dietary treatments initiated at day 40 of gestation and continuing until parturition. Treatments were 60% (restricted, RES), 100% (control, CON), or 140% (high, HIGH) of requirements achieved by controlled intake of complete pelleted diets. At parturition, lambs were removed from dams and fed a common diet consisting of artificial colostrum for the first 20 h followed by ad libitum milk replacer until necropsy on d 20. At necropsy the femur bones were collected. Determinations of bone mineral density (BMD) of isolated femurs were done by dual energy X-ray absorptiometry (DEXA) and peripheral quantitative computed tomography (pQCT). Tomographic measurements were performed for total slice as well as for the cortical and trabecular tissues of the distal metaphysis (23%) and for total slice and the cortical tissue in middiaphysal (50%) sections of the bone. **Results:** The greatest mineralization of the cortical area of the bones, expressed as total bone mineral content (BMC) and total volumetric bone mineral density (vBMD) in the middiaphiysis, was observed in offspring from ewes receiving the CON level of nutrition during pregnancy (1039.7 ± 6.6 vs. HIGH 1013.8 ± 7.5, and RES 1027.2 ± 8.6; $P<0.06$). In the distal metaphysis the mean cortical thickness [mm], cortical content [mg/mm], and cortical area [mm^2] was reduced in ewes receiving RES vs. CON and HIGH treatments (0.63 ± 0.02, 0.73 ± 0.02, 0.73 ± 0.03, $P<0.03$; 27.5 ± 0.1, 31.8 ± 0.1, 31.1 ± 1.7, $P<0.05$, and 33.7 ± 1.1, 38.8 ± 1.1, 38.1 ± 2.0; $P<0.04$ respectively). Bending strength-strain index, allowing for prediction of mechanical properties of the cortical bone within the distal metaphysis indicate weaker bones in lambs from RES vs. CON (90.1 ± 3.8 vs. 104.5 ± 3.9; $P<0.05$) ewes. Within trabecular bone the observed changes indicate that total bone density [mg/cm^3] was reduced in lambs from RES vs. CON (269.3 ± 8.9 vs. 287.7 ± 4.4; $P<0.01$) ewes and increased in those from HIGH vs. CON (287.7 ± 4.4 vs. 300.1 ± 6.6; $P<0.01$) ewes. **Conclusions:** Both restricted and high maternal nutritional planes negatively affected longitudinal bone growth and composition. Data indicate that changes in plane of nutrition during gestation affect bone development, and therefore maintaining an adequate maternal nutritional has the potential to improve the quality of bone tissue and maintain adequate bone growth and development of the offspring during early postnatal development. *Supported by USDA-NRI grant 2005-35206-15281 to JSC, KAV, and DAR.*

MATURATION OF CARDIOMYOCYTES IS CONTROLLED BY THYROID HORMONE

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Objectives: Maturation of cardiomyocytes in the fetus involves transition from a proliferating to a terminally differentiated non-proliferating state. There is a pre-partum increase in circulating thyroid hormone (T_3), a key regulator of organ development and maturation. We have previously shown T_3 administration *in vitro* significantly decreases proliferation of late gestation fetal sheep cardiomyocytes (Chattergoon *et al.*, 2007). In our current study, we hypothesize that T_3 drives maturation of fetal cardiomyocytes by decreasing their proliferative capacity and that the expression of cell cycle regulators involved in cardiac maturation are under the control of thyroid hormone. **Methods:** Homogenates were prepared from snap-frozen ovine left ventricular (LV) tissue of four different ages: 95 days gestational age (dGA; term is 145d GA), 135dGA, neonatal (1 week postnatal), and adult (female); these tissues were from control animals (n= 5). An additional group of T_3-infused fetuses were studied to determine the effects of T_3 on cardiac cell cycle markers in the heart; fetal sheep were infused with T_3 (54μg/day) for 5 days (125-130dGA), prior to onset of the normal prenatal T_3 surge. All animals were euthanized by intravenous overdose of sodium pentobarbital. Hearts were excised, weighed and a section of the LV was frozen for molecular analysis. Cell cycle markers (p21, cyclin D1) and thyroid receptor (alpha 1, beta 1) expression were measured by western blot analysis (n= 6). **Results:** p21 expression was negligible in fetal hearts but increased in the postnatal period. In contrast, cyclin D1 expression increased with gestation but decreased postnatally; expression in adult hearts was only 25% of that in fetal hearts. Near-term hearts had more than 3 times the expression of thyroid receptors as found at the other ages studied. Thyroid hormone infusion caused a significant increase in p21 expression and a significant decrease in cyclin D1 protein levels. There was no change in thyroid receptor alpha 1 but thyroid hormone infusion caused a sharp decrease to negligible protein levels of thyroid receptor beta 1. **Conclusions:** The ontogenic profile of cardiac cell cycle protein expression corresponds with the cardiomyocyte proliferation patterns; p21 is lowest and cyclin D1 is highest at a time where proliferation is occurring (i.e. the fetal period). Thyroid hormone increases p21 and decreases cyclin D1 expression. This is consistent with an acceleration of the normal ontogenic expression of these proteins, thus T_3 is a potent regulator of cardiomyocyte proliferation and maturation. High concentrations of T_3 at an early period of gestation may lead to premature maturation of the myocardium, and ultimately result in fewer cardiomyocytes at birth.

PROGRAMMED HYPOTHALAMIC METABOLIC SENSORS: MECHANISMS FOR HYPERPHAGIA AND ADULT OBESITY IN OFFSPRING OF MATERNAL UNDER- AND OVER-NUTRITION

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Objectives: Exposure to either under-nutrition or over-nutrition in early life increases the risk of adult metabolic syndrome. We have shown that both offspring of maternal food restriction and maternal obesity exhibit hypoleptinemia at birth, and hyperphagia and adult obesity. Central hypothalamic appetite regulation involves nutrient/metabolic sensors**,** (NAD-dependent deacetylase**,** SIRT1 and mammalian target of rapamycin, mTOR. Both, mTOR and SIRT1 colocalize with neuropeptide Y and proopiomelanocortin neurons in the arcuate nucleus. Fasting induced hypothalamic SIRT1 regulation is abnormal in leptin-deficient obese mice, and inhibition/silencing of hypothalamic SIRT1 decreases food intake and body weight gain. Hypothalamic mTOR activity is increased by leptin, and inhibition of mTOR signaling blunts leptin's anorectic effect. Therefore, we determined the hypothalamic expression of SIRT1 and mTOR in FR and HF newborns. **Methods:** At 3 week of age, female rats were weaned to high fat (HF: 60% k/cal) or (control, 10% k/cal) diet. At 11 weeks of age, these rats were mated and continued on their respective diets during pregnancy. An additional group of dams were 50% food-restricted from pregnancy day 10 to term (FR). Hypothalamic protein expression (Western Blot) was analyzed. Values were normalized to GAPDH and presented as fold change. **Results:** At 1 day of age, FR newborns showed significantly increased hypothalamic SIRT1 expression (1.4-fold) with comparable mTOR expression as the Controls. In contrast, HF newborns had unchanged SIRT1 though significantly decreased mTOR expression (0.6-fold). **Conclusions:** The hyperphagia seen in FR and HF newborns is mediated by different metabolic sensor and signaling pathway; in FR newborns, upregulated SIRT1 whereas in HF downregulated mTOR contributes to hyperphagia.

PROGRAMMED SIRT1/PGC1-MEDIATED MECHANISM FOR NEONATAL NON-ALCOHOLIC STEATOHEPATITIS IN OFFSPRING EXPOSED TO MATERNAL OBESITY

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Objectives: Obesity and metabolic syndrome are associated with non-alcoholic fatty liver. SIRT1, a nutrient sensor, regulates hepatic lipid homeostasis via transcriptional co-activator (PGC-1). Activation of SIRT1 protects from diet-induced obesity and metabolic abnormalities. We have shown that maternal obesity and high fat (HF) diet prior to and during pregnancy results in normal birth weight newborns. When nursed by HF dams, these offspring (HF) demonstrate early onset of obesity and lipid abnormalities. We hypothesized that hepatic lipid content of HF offspring would reflect a primary lipid dysfunction. We determined the hepatic lipid content and expression of SIRT1, PCG-1 and its downstream targets involved in fatty acid oxidation (PPARα), lipogenesis (SREBP1) and gluconeogenesis (HNF4α). **Methods:** HF dams were fed a high fat diet (60% k/cal) from 3 weeks of age, mated at 11 weeks, and maintained on the HF diet throughout pregnancy and lactation. Control group were fed ad libitum laboratory chow (10% k/cal). All offspring were nursed by their own dams. At 3 weeks of age, hepatic triglyceride content and protein expression (Western Blot) were analyzed. **Results:** At 3 weeks of age, HF males had significantly increased hepatic triglyceride (260 ± 10 vs. 159 ± 13 mg/g liver). However, hepatic SIRT1 (1.8-fold), PCG1α (1.5-fold) and PGC-1β (1.7-fold) were upregulated. Notably, HNFα (0.6-fold) and PPARα (0.5-fold) were down-regulated whereas SREBP1 (2.1-fold) was upregulated. **Conclusions:** In the HF offspring, fatty liver results from reduced fatty acid oxidation (PPARα) and enhanced lipogenesis (SREBP1). The paradoxical SIRT1 and PGC-1 up-regulation and PPARα/HNF4α down-regulation suggest dysregulated signaling and/or epigenetic modification.

DIFFERENTIAL REGULATION OF ADIPOGENIC TRANSCRIPTION FACTOR (PPARΓ) IN NEWBORNS EXPOSED TO MATERNAL OBESITY AND MATERNAL UNDER-NUTRITION

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Objectives: Exposure to either under-nutrition or over-nutrition in early life results in offspring which exhibit adult obesity. Increased adiposity may be mediated via upregulation of adipogenic transcription factor, PPARγ, which promotes adipocyte differentiation and lipid storage. PPARγ transcriptional activity is repressed by co-repressor complexes (SIRT1 and NCoR/SMRT) which bind to promoter regions of PPARγ target genes and inhibit transcription.. Conversely, co-activators SRC1/TIF2 directly activate PPARγ-mediated transcription. We determined the protein expression of PPARγ and co-repressors/co-activators. **Methods:** At 3 week of age, female rats were weaned to high fat (HF: 60% k/cal) or (control, 10% k/cal) diet. At 11 weeks of age, these rats were mated and continued on their respective diets during pregnancy. An additional group of dams were 50% food-restricted from pregnancy day 10 to term (FR). Newborns were delivered spontaneous, sacrificed at day one of life, and adipose tissue protein expression analyzed (Western Blot). **Results:** In both normal birth-weight (HF) and growth restricted (FR) newborns PPARγ levels were significantly upregulated (2-fold). In HF newborns, co-repressors were downregulated (SIRT1, 0.5-fold; NCoR, 0.4-fold; SMRT, 0.6-fold) with unchanged TIF2. In contrast, in FR newborns co-repressors were upregulated (SIRT1, 1.5-fold; SMRT, 2.6-fold) while the co-activator SRC1 (2.3-fold) was upregulated. **Conclusions:** The underpinning contributory factor to enhanced adipogenesis in both HF and FR newborns is upregulated PPARγ. However, PPARγ activity is enhanced under limited or excess nutrient availability via different mechanisms: HF-mediated downregulation of co-repressors versus FR-mediated upregulation of co-activators. Therapeutic interventions for the prevention of offspring obesity will require target-specific modalities dependent upon the primary etiology.

MATERNAL CREATINE PRE-TREATMENT PROTECTS THE NEWBORN BRAIN AND DIAPHRAGM FROM HYPOXIC INJURY

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Objectives: Using a model of intrapartum hypoxia in the precocial spiny mouse, we have previously shown that a maternal diet supplemented with 5% creatine monohydrate from mid-pregnancy improves survival and postnatal growth in offspring. This study assessed the potential for maternal creatine supplementation to protect the fetal brain and diaphragm from intrapartum hypoxia. **Methods:** Pregnant spiny mice were fed a control or 5% creatine-supplemented diet from day 20 of gestation (term ~39 days). On day 38, pups were delivered by caesarean section, or intrauterine hypoxia was induced by placing the excised uterus containing all fetuses in a saline bath for 7.5-8mins, after which the fetuses were expelled and resuscitation attempted by manual palpation of the chest. Surviving neonates were cross-fostered to a nursing dam for 24h. At post mortem fetal brains were immersion fixed and processed for BAX and caspase-3 immunohistochemistry. Diaphragm muscle was also collected and pinned onto dental wax at the approximate *in vivo* length, and stored in a relaxing solution containing glycerol at -20°C until use. Single fibres were then isolated by dissection, chemically 'skinned' (membrane removed), and Ca^{2+}- and Sr^{2+}-activation profiles obtained. Samples were also snap frozen and stored at -80°C to determine myosin ATPase, NADH+ and succinate dehydrogenase activities. **Results:** Birth-hypoxia caused significant mortality, and 24 h after birth the brains of surviving offspring showed significant increases in lipid peroxidation, and the number of cells expressing the pro-apoptotic protein BAX and cytoplasmic cytochrome c in the cortical subplate, thalamus and piriform cortex. In the diaphragm, hypoxia caused a significant decrease in fibre cross-sectional area, maximum force of contraction, and increase of mRNA levels of the muscle mass-regulating genes, MuRF1 and myostatin. When pregnant dams were fed the creatine-supplemented diet, there was improved postnatal survival and growth of pups, and the increase in markers of brain and diaphragm injury were prevented so that they were not different from caesarean-delivered neonates. **Conclusions:** This study shows that maternal creatine loading before birth significantly protects the fetal brain and diaphragm from hypoxia-induced damage at birth, and may protect against the cortical and white matter injury evolving after birth as a result of intrapartum hypoxia. We suggest that creatine should be considered a promising prophylactic therapy for pregnancies classified as high-risk for fetal hypoxia.

EXCESS MATERNAL GLUCOCORTICOIDS DURING MID-GESTATION: SEXUALLY DIMORPHIC CONSEQUENCES FOR MANY ORGAN SYSTEMS

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Objectives: Excess maternal glucocorticoids (GC) are known to cause significant developmental challenges for the fetus, but these can vary depending on the sex of the offspring. We recently developed a model of acute exposure to excess GC in pregnancy using the spiny mouse. Treatment with dexamethasone (Dex) was given at mid-gestation (day 20-23, term is 39 days), at the pre-glomerular stage of kidney development in this species, a time when kidney development is known to be vulnerable to such insults. This is also the point in time when the labyrinth region of the placenta develops. We have previously shown that offspring from Dex-treated dams have a reduced nephron number, driven at least in part by a reduction in key branching genes in the fetal kidney, and adult male offspring respond poorly to surgical stress. Here we present data from the placenta, heart and adrenal gland of offspring from saline and Dex-treated dams. **Methods:** Dex (125μg/kg) or saline was administered to the dam by osmotic pump for 60h from gestation day 20. Heart (d23 & 37GA, 20wks PN), placenta (d23 & 37GA), and adrenal (20wks) were collected. mRNA expression of *Igf1*, *Igf1r* was analysed via qPCR within the hearts. Proportions of labyrinth (Lab) and junctional zone (JZ) within the placenta were analysed histologically and mRNA expression of *Bax*, *Bcl2*, *Vegfa*, *Vegfr2*, *Gcm1* and *Map2k1* determined. Adrenals were processed for p450c17 and cytochrome b5 immunohistochemistry. **Results:** Within the placenta dex reduced the Lab:JZ ratio at d23 and led to an increase in *Map2k1* and *Vegfa*, with a greater increase of *Map2k1* in females. The *Bcl2:Bax* ratio was increased in response to dex at d23, more so in females. *Igf1* increased 2x between 23 & 37 dGA in control fetal hearts, this increase was suppressed with dex exposure, and hence I*gf1* was lower on d37 in dex-exposed hearts. *Igf1r* was 6x ($p<0.001$) and 2x higher on d23 & 37, respectively, for dex-exposed fetal hearts. The expression of p450c17 in the zona reticularis of the adrenal gland was decreased in dex-exposed males. Cytochrome b5 expression was decreased throughout the adrenal cortex in male and female dex-exposed offspring. **Conclusions:** Excess maternal GC have organ, gene and sex-specific consequences for the fetus and offspring. Here we have shown sex-specific changes in gene and protein expression in the placenta, heart and adrenal gland following maternal dex exposure. The consequences of these gene/protein changes on the structural integrity, and adult function, of these organs remains to be completely elucidated. Overall this model has highlighted the sensitivity of a range of fetal organs at mid-gestation to excess maternal GC. Ongoing experiments are examining the structure of these organs, as well as looking at other organs such as the liver and brain.

THE SPINY MOUSE–AN IDEAL SPECIES TO STUDY PERINATAL BIOLOGY

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Objectives: The spiny mouse is a precocial rodent species native to regions of the Mediterranean. We suggest that it is an ideal species for the study of perinatal biology, based on some key features of pregnancy. The major circulating glucocorticoid in the spiny mouse is cortisol, and the adrenal gland produces DHEA. The gestation length is 39 days, and litter sizes range from 1-5 (mean is 2-3). The placenta of the spiny mouse is hemotrichorial and the labyrinth (fetal zone) is present from mid-gestation, occupies the majority of the placental area from day 28 of gestation and vascularisation is an ongoing process until term. Parturition is followed by a post-partum oestrus in the dam, such that conception of the next litter occurs within 24h of delivery. The ovary, via an active corpus luteum maintains pregnancy, however the mechanisms of labor are unknown. Spiny mouse offspring are born at an advanced stage of development, with locomotion and thermogenesis occurring from the day of birth. Maturation is rapid, with adrenache occurring at postnatal day ~15 and sexual maturity reached at 3 months of age. We have described the stage of development around the time of birth of several organs in the spiny mouse. Specifically, nephrogenesis is completed in the kidney at day 38 of gestation. The adrenal medulla is present from day 35 of gestation, with zonation of the adrenal gland complete at day 2 postnatally. The most rapid period of brain growth occurs from day 30-35 of gestation with myelination being an active process from day 35 of gestation. The major immune organs (spleen, bone and thymus) establish adult-like structure from day 34 of gestation. In the lung secondary septal crests are observed from day 30 of gestation and reach a peak at day 14 postnatally. The diaphragm undergoes significant remodelling between days 35 and 38 of gestation in preparation for continuous breathing at birth; fetal breathing movements can be observed by ultrasound from day 29 of gestation. In parallel with the ontogeny studies described above we have used the spiny mouse to study several conditions important to human perinatology, such as birth asphyxia, maternal stress, and maternal viral infection.

HYPOXIA-INDUCED APOPTOSIS IN THE FETAL GUINEA PIG FOREBRAIN IS MEDIATED BY NITRIC OXIDE

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Objectives: Hypoxia-induced apoptosis in fetal brains has been shown to cause neuronal injury. Hypoxia and nitric oxide (NO) are important regulators of apoptosis. We have previously shown that chronic hypoxia increases NO synthesis in fetal left cardiac ventricles and decreases apoptosis in an NO-dependent manner via upregulation of inducible nitric oxide synthase (iNOS). NO has also been shown to both induce and inhibit apoptosis, depending on the conditions of study. Thus the aim of this study is to quantify the effect of hypoxia on stimulating apoptosis in the fetal forebrain and determine the role of iNOS-derived NO in mediating the apoptotic response. Caspase 3 activity and DNA fragmentation are used as indices of apoptosis. **Methods:** Pregnant guinea pigs were exposed to room air (normoxic, NMX) (N=5) or 10.5% O_2 (hypoxic, HPX) (N=5) for 14d prior to term (term=65d). L-N6-(1-Iminoethyl)-Lysine (L-NIL), a selective iNOS inhibitor, was administered to pregnant mothers via their drinking water at a dose of 1-2mg/kg/d while control animals received only water. At 60d gestation, near term fetuses were removed via hysterotomy from anesthetized sows and fetal brains extracted. Forebrains were rapidly frozen in liquid N_2, and stored at -80ºC. Caspase 3 activity and DNA fragmentation were measured with commercially available assay kits. **Results:** Chronic hypoxia increased (P=0.022) caspase 3 activity by 30% compared to normoxic controls (132.27±9.9 vs 172.24±2.8 pmol AMC/min/mg protein, NMX vs HPX). L-NIL prevented the hypoxia-induced increase (P=0.036) in caspase 3 activity to levels similar to normoxic values (129.26±9.9 pmol AMC/min/mg protein, HPX+L-NIL). In the same tissues, chronic hypoxia increased (P=0.006) DNA fragmentation by 80% compared to normoxia (0.043±0.007 vs 0.077±0.007 OD value $_{405nm}$/mg protein, NMX vs HPX). L-NIL prevented the hypoxia-induced increase in DNA fragmentation to levels similar to normoxic controls (0.021±0.002 OD value $_{405nm}$/mg protein, HPX+L-NIL). **Conclusions:** These results indicate that chronic intrauterine hypoxia induces apoptosis in the fetal guinea pig forebrain as measured by an increase in caspase 3 activity and DNA fragmentation. NO plays an important role in stimulating apoptotic mechanisms in hypoxic fetal forebrains. We conclude that hypoxia-induced cell injury may be mediated by pro-apoptotic proteins that are NO dependent. [(NIH HL90044/LE) & NIH HL49999/LT)]

EFFECTS OF MATERNAL DIET ON FETAL OVARIES: CELL PROLIFERATION, VASCULARIZATION, APOPTOSIS AND GAP JUNCTIONS

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Maternal diet during pregnancy affects numerous developmental processes in the growing fetus and also affects organ systems in the offspring from the neonatal stage to adulthood; this phenomenon is termed "developmental programming." The aim of the present study was to determine if differing levels of energy and Se in the maternal diet impact fetal ovarian size and function, as measured by cellular proliferation, vascularization, apoptosis and expression of gap junctional connexin (Cx) proteins. Sheep (n=26) were fed maintenance (M; 2.12 Mcal/kg) or energy-restricted (R; 60% of M) diets with high Se (HSe; 81.5 μg/kg body weight) or adequate Se (ASe; 7.4 μg/kg body weight) from 21 days before breeding to day 135 of pregnancy (day of tissue collection). At collection, fetuses were weighed, and fetal ovaries were weighed and fixed. Ovarian sections were stained immunohistochemically for the presence of proliferating cell nuclear antigen (PCNA; a marker of proliferating cells), factor VIII (a marker of endothelial cells and thus vascularization), apoptotic cells (TUNEL method) and Cx26, 37 and 43, followed by image analysis. Maternal R diet but not level of Se decreased the weight of fetuses and ovaries. Labeling index (LI; proportion of proliferating cells) was similar for theca cells (TC) and granulosa cells (GC), and for secondary (SF) or antral follicles (AF), and was greater for SF and AF than for primordial (PR) or primary (P) follicles. The R diet and/or HSe affected LI in all follicle types, in stromal tissues and in blood vessels (BV). A dense network of BV was detected in the areas containing SF to AF and in the medulla and hilus, but areas containing PRF were poorly vascularized. The number of apoptotic cells was minimal. Cx26 was localized to TC of SF, early AF and AF, and to BV and stroma; Cx37 was localized in an area between the oocyte and the GC of PR, P, SF and AF, and in endothelial cells of BV; Cx43 was localized in the GC of PR, P, SF and AF, to the TC of AF, and in an area between the oocyte and the GC in all follicle types. Cx26 expression was greater in AF than early AF, and the R diet decreased Cx26 expression in AF. Cx43 expression was greater in GC of AF than those of P or SF, and was greater in GC than TC of AF. Maternal diet did not affect Cx43 expression in P or SF; but for AF, Cx43 expression was greater in ewes fed the M diet with HSe than in any other treatment groups. These results demonstrate that for fetal ovaries: 1) maternal diet affected cell proliferation in all compartments; 2) the majority of ovarian compartments are densely vascularized, except cortex; 3) apoptosis is minimal; 4) Cx26, 37 and 43 are expressed, but their localization differs; and 5) maternal diet affected Cx26 and Cx43 expression in AF. These results emphasize the importance of maternal diet in growth and development of fetal ovaries. *Supported by USDA-NRICGP, 2005-35206-15281; P20 RR016741, INBRE of the National Ctr. for Res. Resources, and ND Hatch Project ND01712.*

AUTOMATED DETECTION AND ANALYSIS OF FLUORESCENT BIOMARKERS IN HUMAN PLACENTAL CHORIONIC TISSUE

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Objectives: Functional analysis of receptors mediating transport or signal transduction at the materno-fetal barrier is based on their *in-situ* allocation to cellular compartments of the chorionic tissue (e.g. syncytiotrophoblast; STB). Measurements of changes in location or levels of expression due to different genotypes and environmental exposures can help to understand mechanisms of pregnancy-associated diseases such as preeclampsia. Therefore, multimolecular analysis of chorionic tissue compartments in combination with exhaustive bioinformatic knowledge extraction (cytomics) is of growing interest. Cytomics-technologies are often microscope-based using automated *in-situ* identification of cellular systems (tissue areas, cells, subcellular structures) and linear quantification of associated molecules. Currently available software does not support the *in-situ* analysis of placental chorionic tissue with its complex shapes/textures of villi, multinuclear STB or highly autofluorescent nuclear-free erythrocytes (erys). Our aim is to develop a new approach called holistic pattern-recognition for detection of such structures. **Methods:** Paraffin-sections of placental chorionic tissue were labeled with target-specific primary and fluorescent secondary antibodies. Digital fluorescence- or transmitted light-images were acquired with a multichannel immunofluorescence microscope with motorized stage controlled by TissueFAXS software. Automated recognition of STB/villi by cytokeratin7 expression or erys detection by shape identification was done combining classical digital image-processing and pattern recognition approaches with state of the art machine-learning techniques. **Results:** We demonstrate the applicability of our approach to automatically identify *in-situ* placental chorionic villi and STB areas in high correlation (>90%) with two human experts. Any fluorescent-labeled protein of interest can subsequently be allocated to these structures and fluorescent pixels associated with these areas can be linearly quantified after virtual subtraction of interfering background fluorescence caused by maternal and fetal erys. This is exemplified by automated analysis of the location of the receptor for advanced glycated end products (RAGE) in 9 x 9 fields of view/placenta from human healthy chorionic tissue. In addition, since alteration in RAGE expression has been recently associated with preeclampsia, the chorionic allocation and level of RAGE expression is compared between 12 preeclamptic and age-matched control placentae. **Conclusion:** A novel approach for automated analysis of immunofluorescent-labeled chorionic tissue is presented. The applications of this method lie in analyses of protein allocation and automated linear quantification of markers in placental chorionic tissue to study placental protein function in health and disease.

2-HYDROXYESTRADIOL AND 4-HYDROXYESTRADIOL INDUCE PROLIFERATION OF UTERINE ARTERY ENDOTHELIAL CELLS: ROLE OF B-ADRENERGIC RECEPTORS, P38 AND P42/44 MITOGEN-ACTIVATED PROTEIN KINASES

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Introduction: We recently reported that physiologic levels of estradiol-17β ($E_2\beta$) and the catecholestradiols 2-Hydroxyestradiol (2-OHE_2) and 4-Hydroxyestradiol (4-OHE_2) induce pregnancy-specific uterine artery endothelial cell (UAEC)proliferation. However, unlike $E_2\beta$, the proliferative effects of its catechol metabolites were not mediated via estrogen receptors (ER) ER-α or ER-β. Thus, the precise mechanism of action of catecholestradiols on UAEC mitogenesis remains to be elucidated. Catecholestradiols have close structural similarities to catecholamines and have affinity for adrenergic receptors (AR). Therefore, we **hypothesized** that the catecholestradiols stimulate UAEC proliferation via α-ARs and/or β-ARs and a G-protein-coupled receptor signaling pathway through mitogen-activated protein kinases (MAPK). **Methods:** Validated UAECs from late pregnant sheep (day 120-130; term = 147 d; n=5) were pretreated (10μM; 1 hr) with the nonselective α-AR blocker phentolamine or nonselective β-AR blocker propanolol or SB203580 (p38 MAPK inhibitor), or PD98059 (p42/44 MAPK inhibitor) followed by treatment with vehicle, 0.1 nM or 100 nM 2-OHE_2 or 4-OHE_2 for 24 hours. An *in vitro* index of proliferation was evaluated utilizing the Brdu Proliferation Assay technique. Identification of AR subtypes (α_1, α_2, β_1, β_2 and β_3) in UAECs was performed by Western Blotting. Temporal activation of MAPKs via phosphorylation was evaluated utilizing Immunoblotting. **Results:** Western Blotting revealed the presence of α_2, β_2 and β_3 AR in UAECSs; however, α_1 and β_1 were not detectable. Both 2-OHE_2 and 4-OHE_2 treatment significantly increased (P<0.01) UAEC mitogenesis. Pretreatment with the α-AR antagonist phentolamine did not alter UAEC proliferation response to 2-OHE_2 and 4-OHE_2. In contrast, the β-AR blocker propanolol, the p38 MAPK inhibitor, and the p42/44 MAPK inhibitor pretreatments completely abrogated (P<0.01) the proliferative effects of the catecholestradiols. Western Blotting revealed a time course-specific activation of phosphorylated p38 and p42/44 MAPK. **Conclusions:** These data demonstrate that catecholestradiols stimulate proliferation of UAECs via β_2 and β_3 AR but not α-ARs. Moreover, catecholestradiols directly activate G-protein-coupled receptor signaling pathways that converge at p38 and p42/44 MAPK signaling to trigger proliferation. Our findings indicate that physiological approaches to investigating endothelial proliferation and pregnancy-induced angiogenesis require specific appreciation of catecholestradiols and β-adrenergic G-protein coupled receptor signaling though both p38 and p42/44 MAPKs. These data also point to the potential relevance of the convergence of the sympathomimetic system and catecholestradiols in the regulation of pregnancy-induced angiogenesis. ***NIH* HL49210, HD38843, HL87144 and R25GM083252**

ALTERED GENE EXPRESSION IN PLACENTA DURING DIABETIC PREGNANCY: MODULATION BY MATERNAL DIET

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Diabetes during pregnancy is a known risk factor for congenital malformations, as well as adverse pregnancy outcomes, including predisposition to disease later in life, such as metabolic syndrome. We hypothesized that maternal diabetes might contribute to adverse outcomes through aberrant regulation of gene expression during placental development. **Objective:** To investigate placental development under conditions of maternal diabetes with focus on altered gene expression. Methods: We applied a combination of genome-wide expression profiling, targeted quantitative measurements and in situ hybridization to analyze placental gene expression at midgestation in a mouse model of diabetes. Histological and morphometric studies were used to define effects of maternal diabetes on placental development. **Results:** More than a hundred genes exhibited deregulated gene expression in the diabetic placenta, many of them not previously implicated in placental development. Alterations were observed in both the embryonic and maternally derived compartments. The reduced growth of the placenta in diabetic pregnancies was associated with a reduction of the size of the junctional zone and the labyrinth, and with aberrant spongiotrophoblast differentiation. Intriguingly, moderate changes in composition of the maternal diet were able to modulate growth, as well as the severity and the timing of altered gene expression in diabetic placenta. **Conclusions:** Metabolic imbalance in the mother affects specific pathways in tissue- and cell-type-specific manner. Diabetes-induced alterations in placental gene expression precede aberrant cell differentiation; this, together with the reduced growth of the placenta under diabetic conditions may impact placental function. Although adverse and beneficial components remain to be identified, maternal diet composition is an important factor in modulating the impact of diabetic conditions during pregnancy. Our findings may have relevance for placental insufficiency in diabetic pregnancies as well as for nutritional epigenomics and developmental programming. Supported by RO1-HD037804 to C. Kappen and RO1-HD055528 to J.M. Salbaum.

MATERNAL RECOGNITION OF PREGNANCY PROGRAMS EARLY ALTERATIONS IN OVINE UTERINE ENDOTHELIAL CALCIUM AND NITRIC OXIDE REGULATION

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Maternal recognition of pregnancy is a critical period associated with many signals and physiologic changes required to maintain pregnancy. In ewes, the rise and timing of the elevation in uterine blood flow (UBF) is critical to the survival of the embryo and without this rise, pregnancy is lost. The mechanism by which these early rises in UBF occur is unknown and is the aim of this research. Previously we reported characteristic changes in uterine artery endothelium during late gestation, including rises in: 1) Nitric Oxide (NO) production; 2) eNOS expression; 3) level of eNOS phosphorylated at stimulatory sites in excess of total eNOS levels; and 4) upon stimulation with ATP rises in the frequency of calcium bursts that are critical to maintain sustained NO production. We hypothesized that during Maternal Recognition of Pregnancy uterine artery endothelial cells (UAEC) begin to function more like late pregnant UAEC and NO from these cells may play a role in this early conceptus-induced rise in UBF. Ewes were either in the control nonpregnant (NP) group or an early pregnant (EP) group on day 14 or 16 of pregnancy. At surgery, UBF was measured, ewes were, ovariohysterectomized and pregnancy status confirmed. Uterine arteries were obtained, noting the branch generation which were used for the subsequent Fura-2 calcium imagining experiments (3^{rd} generation) and the endothelium isolation for eNOS expression (2^{nd}-3^{rd} generation) UBF was increased during EP and was greater than NP by day 16 of pregnancy; NP control, day 14 and day 16 (16.1±2.0, 30.1±10.9, and 39.0±5.7 ml/min, respectively). In EP vessels eNOS expression was elevated in day 16 compared to luteal and day 14 EP (0.47±0.08, 0.24±0.04 and 0.19±0.03 OD, respectively). *Ex vivo* cellular imaging of uterine arteries, which upon stimulation with ATP allows for direct real-time imaging of calcium within individual UAEC, showed that the number of calcium burst per endothelial cell at 30 min was greater in day 16 pregnant compared to the NP controls, with day 14 pregnant vessels intermediate to day 16 pregnant and NP controls, but still elevated. Previous reports from our laboratory also using ATP show that compared to NP sheep, UAEC from late pregnant ewes also have increased numbers of calcium bursts per cell which appear to be similar to the day 16 EP calcium responses we report herein. The increase in the number of ATP-associated calcium bursts and increasing eNOS expression demonstrates that even as early as maternal recognition of pregnancy the uterine vasculature is being programmed to a vasodilator producing phenotype. Programming of the uterine vasculature early in pregnancy maybe a key component to the rise in UBF associated with maternal recognition of pregnancy. Furthering our understanding of the mechanisms that lead to rise in UBF and programming of these vessels will further our understanding of EP loss at such a critical window of gestation. *NIH HL49210, HL87144, and HD38843.*

THE SP1 TRANSCRIPTION FACTOR IS CRUCIAL FOR THE ESTABLISHMENT OF GLUCOCORTICOID BARRIER IN HUMAN PLACENTA

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Objective: Over-exposure of the fetus to glucocorticoids during gestation is not only detrimental to fetal growth and development, but also thought to program the development of adult diseases. The glucocorticoid level in the fetal circulation is normally kept about 10 times lower than the maternal circulation during gestation. This glucocorticoid concentration gradient is established and maintained by the presence of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) in the placental syncytiotrophoblasts, where this enzyme converts cortisol to biologically inactive cortisone. However, as the progenitor of syncytiotrophoblasts, cytotrophoblasts express very little 11β-HSD2. Here we studied the mechanism underpinning this increase of 11β-HSD2 expression upon syncytialization by measuring the methylation status of the CpG-rich 11β-HSD2 promoter as well as identifying the transcription factor mediating the effect of the hCG/cAMP pathway, which has recently been shown to play an important role in maintaining syncytial 11β-HSD2 expression. **Methods:** Freshly isolated human term placental cytotrophoblasts and cultured syncytiotrophoblasts were used. Real time PCR and Western blotting were conducted to measure gene expression. Bisulfite sequencing was utilized to determine the methylation status. Chromatin immunoprecipitation (ChIP) was used to assay the proteins bound to the promoter. **Results:** The expression of 11β-HSD2 was indeed markedly increased when cytotrophoblasts fused to form syncytiotrophoblasts. Although one CpG site at -22 bp was found to be methylated in both cytotrophoblast and syncytiotrophoblast, the other CpGs were found to be unmethylated in either cell types. Since the 11β-HSD2 gene promoter carries multiple binding sites of Sp1, we investigated the expression profile of Sp1 during syncytialization as well as upon the activation of the cAMP/PKA pathway. We found that Sp1 expression was markedly increased during syncytialization, which correlated with 11β-HSD2 expression. Stimulation of the cells with forskolin or dibutyl cAMP significantly increased Sp1 expression. Knocking-down of Sp1 expression with siRNA or Sp1 antagonist mithramycin greatly attenuated not only the basal expression of 11β-HSD2 but also forskolin or dibutyl cAMP-stimulated expression of 11β-HSD2 in the syncytiotrophoblasts. ChIP demonstrated that stimulation of the syncytiotrophoblasts with forskolin or dibutyl cAMP increased the bindings of Sp1 and polymerase II to 11β-HSD2 promoter. In the mean time, the acetylation level was increased and the methylation level was decreased in the histone 3 (H3K9) associated with 11β-HSD2 promoter. **Conclusions:** The increase of 11β-HSD2 during syncytialization is at least in part due to the increased expression of Sp1 upon activation of the cAMP pathway rather than the differential methylation status of the 11β-HSD2 promoter.

REDUCING NUTRIENT INTAKE OF OBESE EWES TO REQUIRMENTS FROM 28 DAYS OF GESTATION (DG) REDUCES THE EFFECTS OF MATERNAL OBESITY ON CONCEPTUS GROWTH BY MIDGESTATION.

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In the ewe, maternal obesity from conception results in fetal macrosomia and altered organ growth by midgestation. In both pregnant women and ewes, maternal obesity from conception through pregnancy results in increased newborn adiposity and insulin resistance postnatally. **Objectives:** To determine effects of reducing maternal nutrient intake in obese ovine pregnancies in early gestation on fetal growth by midgestation. **Methods:** Multiparous ewes of similar weight (69.9 $\pm$ 1.9 kg) and body condition score (BCS, 5.4 $\pm$ 0.1) and carrying twin pregnancies were randomly assigned to control (C, 100% of NRC requirements, n =8), or obese (OB, 150% of NRC, n=8) groups from 60 days before mating to midgestation, or to an OB-dietary intervention (OB-DINT) group (150 % of NRC from 60 days before mating through 28 dG, then reduced to 100 % NRC through midgestation, n = 8). Ewes were necropsied on 76 $\pm$ 2 dG, and fetal size and weight as well as selected fetal organs weights were collected. Data were analyzed using the GLM procedure of SAS. **Results:** Ewes on the C, OB, and OB-DINT groups gained 8.0 $\pm$ 2.3, 31.1 $\pm$ 2.3, and 36.7 $\pm$ 2.3 %, respectively, from diet initiation through breeding. At midgestation, OB ewes had gained more weight ($P<0.05$) than OB-DINT ewes (59.4 $\pm$ 3.0 vs. 48.9 $\pm$ 3 %), and both groups had gained more weight than C ewes (18.7 $\pm$ 3.0, $P<0.01$). Midgestation fetal weights were similar between C and OB-DINT fetuses (229.3 $\pm$ 13.1 and 235.0 $\pm$ 12.1 g respectively) but was less than that of OB fetuses (278.1 $\pm$ 10.4 g, $P<0.01$). Heart weight was similar in C and OB-DINT fetuses (1.75 $\pm$ 0.12 and 1.80 $\pm$ 0.12 g respectively) but was elevated in OB fetuses (2.28 $\pm$ 0.10 g, $P<0.01$). While right ventricular weight was elevated in OB- DINT fetuses compared to the C fetuses (0.54 $\pm$ 0.04 vs. 0.45 $\pm$ 0.04, $P<0.05$), both were less than that of OB fetuses (0.68 $\pm$ 0.04, $P<0.01$). Liver weight was elevated in OB-DINT fetuses compared with C fetuses (16.3 $\pm$ 0.8 vs. 14.0 $\pm$ 0.8 g respectively, $P<0.01$), but liver weights of both groups were less than in OB fetuses (18.8 $\pm$ 0.8 g, $P<0.01$). Pancreas (0.53 $\pm$ 0.04 vs 0.29 $\pm$ 0.05 and 0.33 $\pm$ 0.04 g, respectively) and perirenal fat (1.51 $\pm$ 0.08 vs.1.00 $\pm$ 0.10 and 0.95 $\pm$ 0.09 g, respectively) weights were greater ($P<0.01$) in OB fetuses vs. C and OB-DINT fetuses. **Conclusions:** Returning dietary intake to requirements in OB ewes from early to midgestation reduced maternal weight and returned fetal size and weight to that observed in C ewes. Additionally, while weights of most fetal organs, including the pancreas were returned to that of C fetuses, weights of other organs (i.e. right ventricle and liver) remained elevated compared to C fetuses. These data demonstrate a marked variation in the sensitivity of fetal organ growth, to dietary interventions that reduce maternal intake during gestation. NIH INBRE P20RR016474.

MATERNAL UTERINE SPACE RESTRICTION INDUCED INTRAUTERINE GROWTH RESTRICTION: EFFECTS ON THE GAP JUNCTION PROTEINS AND ENOS IN OVINE UTERINE ARTERY ENDOTHELIUM

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Introduction: We recently reported that maternal uterine space restriction leads to asymmetric Intrauterine Growth Restriction (IUGR). Models of IUGR exhibit reductions in uterine blood flow and is associated with severely reduced uterine artery endothelial adaptations. Such endothelial adaptations may be modulated by coordinated intercellular interactions via gap junction proteins and eNOS activation. **Hypothesis:** We hypothesized that IUGR associated with maternal uterine space restriction will be directly associated with the expression of the gap junction proteins connexin (Cx)43 and 37 as well as the expression and activity of eNOS in uterine artery endothelium (UA endo) **Methods:** IUGR was created by restricting pregnancy to a single uterine horn (gravid IUGR) via surgically severing the intercorneal vascular connections and then ligating one horn (i.e. contralateral nongravid IUGR) 2-3 months prior to breeding. UA endo was isolated from control nonpregnant sheep (luteal, n=5 vessels; follicular, n=5) as well as late pregnant (120-130d, term = 147d) sheep (nongravid IUGR, n=8; gravid IUGR, n=8, control pregnant n=8). Cx43 and Cx37 protein expression as well as eNOS expression was determined by Western analysis. Functional responses of gap junctions were tested using a Lucifer yellow assay. **Results:** Cx43 and Cx37 were both significantly elevated in control pregnant whereas only Cx43 was significantly elevated in gravid IUGR group compared to luteal. Additionally, Cx43 expression was significantly elevated in the UA endo of the gravid IUGR compared to the nongravid horn whereas Cx37 was barely detectable in both the nongravid and gravid IUGR horns. P^{635}eNOS, and total eNOS showed similar patterns of expression as connexins. Lucifer yellow assays demonstrated functional channels in the uterine artery endothelial cells. The uterine blood flow was significantly lower in the luteal, follicular, and nongravid IUGR groups compared to gravid IUGR and pregnant controls. **Conclusions:** These data demonstrate that IUGR resulting from maternal uterine space restriction is closely associated with maternal uterine gap junction protein expression patterns and their associated eNOS activation state. Both Cx43 and Cx37 are lower in gravid IUGR group compared to pregnant, demonstrating that local responses of fetal restriction to a single horn has a direct effect on connexin expression during pregnancy. It is noteworthy that the gravid IUGR group had lower connexin expression than in the pregnant control horns, further substantiating the plausible role of uterine arterial gap junction proteins in IUGR resulting from uterine space restriction. Finally, the nearly equal magnitude increase in uterine blood flow in both gravid IUGR and pregnant controls may point to compensatory responses to space restriction and/or IUGR. *NIH HL49210, HD38843, HL87144.*

MATERNAL DIETARY PROTEIN LEVEL ALTERS FUNCTION OF LARGE-CONDUCTANCE, CALCIUM-ACTIVATED K (BK_{CA}) CHANNELS IN FETAL CORONARY ARTERIAL SMOOTH MUSCLE CELLS

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Objectives: We previously have shown that maternal nutrient restriction during pregnancy leads to the selective impairment of a novel endothelium-dependent, NO-independent (i.e., endothelium-derived hyperpolarizing factor [EDHF]-like) vasodilator pathway that is mediated via activation of BK_{Ca} channels in fetal coronary arteries (O'Rourke S et al., J. Molec. Cell. Cardiol. 42:S228-S229, 2007). To further examine the mechanisms, we tested the hypothesis that potassium ion conductance thru BK_{Ca} channels is impaired in coronary arteries of fetuses from malnourished ewes. **Methods:** For this study, multiparous, Western whiteface ewes (n=3 per dietary protein level) were fed diets that were isocaloric and isonitrogenous but that differed in metabolizable protein levels (75% [LOW], 100% [moderate, MOD], or 125% [HIGH] of National Research Council recommendations) from day 100 until slaughter on day 130 of gestation (length of gestation approximately 145 days). Freshly isolated coronary arterial smooth muscle cells were obtained at slaughter (n = 5-6 cells per fetus) and were used to determine whole-cell K currents in response to successive voltage pulses of 200 ms duration, increasing in 10-mV increments from -70mV to +50mV in the absence or presence of 100nM iberiotoxin (Ibtx, a selective blocker of BK_{Ca} channels). **Results:** Although fetal weights were 3.35, 4.18, and 2.94 kg (pooled SE = 0.45) for LOW, MOD, and HIGH ewes, respectively, they did not differ statistically ($p>0.23$). Similarly, neither fetal heart weights (22.5, 28.4, and 19.9 g for LOW, MOD, and HIGH; pooled SE = 3.0) nor left or right ventricular wall thicknesses differed ($p>0.21$) between dietary protein groups. In the fetal coronary arterial smooth muscle cells from MOD ewes, Ibtx almost completely inhibited the outward whole-cell K current (72.3 $\pm$ 4.8% inhibition [mean $\pm$ SEM] of peak current density). In contrast, this current was only partially inhibited by Ibtx in fetal coronary arterial smooth muscle cells from LOW or HIGH ewes (30.5 $\pm$ 6.5 and 31.5 $\pm$ 6.2% inhibition of peak current density, respectively). **Conclusions:** These data confirm our hypothesis and suggest that altered maternal protein intake during late pregnancy leads to impaired K conductance through BK_{Ca} channels in coronary artery smooth muscle cells of the fetus, which could explain the dysfunction in the coronary arterial EDHF-like pathway previously observed in offspring of mothers subjected to differing dietary intakes during pregnancy. *Supported by NIH R03-HD061532 to STO, CS, LPR, and JSC, and USDA-NIFA-NRI 2009-35206-05276 to KAV.*

MATERNAL BMI AFFECTS MALE BUT NOT FEMALE PLACENTAL FATTY ACID TRANSPORTERS

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Objectives: Long chain polyunsaturated fatty acids (LC-PUFA) are essential for proper cardiovascular development. The fetus cannot synthesize LC-PUFA, so it depends upon the placenta for delivery. We hypothesized that maternal obesity is associated with altered placental fatty acid transport leading to LC-PUFA deficiency in the offspring of obese women. **Methods:** Women were recruited in their third trimester as part of the *Oregon Women's Study*. At delivery, placental samples and cord blood were collected. Early 1st trimester body mass index (BMI) was calculated from maternal first trimester height and weight. The mRNA levels of placental fatty acid transporters were measured in both groups by Q-PCR. Maternal and umbilical venous fatty acid levels were quantified using gas chromatography-mass spectrometry. **Results:** Maternal levels of LC-PUFA were not significantly associated with BMI. In male fetuses, maternal BMI was significantly and positively associated with the expression of several placental fatty acid transporters (FATP4 (R=0.60); CD36 (R=0.63); FABPpm (R=0.67); $P<0.05$). There were no significant associations between maternal BMI and placental fatty acid transporter expression in female fetuses. The cord vein-to-maternal LC-PUFA fatty acid concentration ratio (indicative of placental fatty acid transport) was negatively associated with maternal BMI only in male fetuses (linoleic acid (C18:2; R= -0.51); α-linolenic acid (C18:3; R= -0.51) $P<0.05$). **Conclusions:** Placental fatty acid transporter expression in male, but not female offspring was related to maternal early pregnancy BMI. This suggests that placentas of male fetuses are more sensitive to maternal body composition than females. Despite the high expression of fatty acid transporters, the male cord vein plasma LC-PUFA concentrations relative to maternal were lower as maternal BMI increased. *The apparent paradox between transporter expression and fetal plasma LC-PUFA profiles must be resolved because adverse LC-PUFA profiles in the plasma of offspring predict unfavorable long term cardiovascular health outcomes.* Support: Northwest Health Foundation, Holtzman Foundation, Audrey Love Foundation, Eagles Trust Foundation.

ESTRADIOL-INDUCED POST-TRANSLATIONAL MODIFICATION OF ENOS IN OVINE UTERINE ARTERY ENDOTHELIAL CELLS FROM LATE PREGNANT SHEEP

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Introduction: *In vivo* studies of unilateral blockade of estrogen receptors (w/ICI 182,780) or nitric oxide synthase (w/L-NAME) locally reduces uteroplacental blood flow during late ovine gestation. Thus establishing a cause and effect relationship between endogenous estrogen in part being responsible for the maintenance of the elevated uterine blood flow (UBF) seen during gestation via the activation of eNOS in the uterine vascular endothelium. We hypothesized that estradiol induces eNOS activation via the non-classical Estrogen Receptor (ER) mediated action and initiates the sequential and coordinated re-partitioning and activation of eNOS with altered multi-site phosphorylation away from the membrane invagination structures known as caveolae. **Methods:** Uterine Artery Endothelial Cells (UAECs) were isolated from uterine arteries of late pregnant ewes. Confluent UAECs were treated estradiol (0.01nM, 0.1nM and 10nM) for 10 minutes and total endothelial cell proteins were obtained. We then treated UAECs with vehicle (Control) or estradiol (10 nM) for 10 minutes from which caveolae were enriched in sucrose density gradient centrifugation. Immunoblotting was utilized to study the multi-site phosphorylation state and re-partitioning of eNOS and ERs from caveolae domains. **Results:** Examining whole cell extracts, we observed an increased phosphorylation of stimulatory ^{P635}eNOS after treatment with all concentration of estradiol. The greatest increase was observed with 10nM (OD=0.15) compared to control (OD=0.04) while 0.01 and 0.1nM had intermediate responses (OD=0.09 and 0.10 respectively). Examining caveolae enrichment experiments, we observed that control UAECs demonstrated that total eNOS was predominantly located in the caveolar domain. In the estradiol-treated UAECs, eNOS was detectable both in the caveolar and non-caveolar domains. In control UAECs, stimulatory ^{P635}eNOS and ^{P1177}eNOS were not detected in any of the fractions whereas inhibitory ^{P114}eNOS was detected predominantly in the non-caveolar domain. In response to estradiol, stimulatory ^{P635}eNOS was detected in all cellular domains whereas stimulatory ^{P1177}eNOS was detected mainly in the caveolar domain. The inhibitory ^{P114}eNOS was decreased by estrogen treatment to low levels in the non-caveolar domain and was not detected in the caveolar domain. In control UAECs, ERs were detected in a higher abundance in the non-caveolar domain and distribution was not altered upon estradiol treatment. **Conclusions:** Estradiol increases phosphorylation of eNOS stimulatory site and produces temporal and spatial re-partitioning of eNOS from the caveolar to non-caveolar domains with altered multi-site phosphorylation state in UAECs. These findings are critical to the understanding of estradiol-induced gestational vascular adaptations. This research was supported by *NIH R25 GM083252, HL49210, HD38843, HL87144, and HL70562.*

THE IMPACT OF FETAL GROWTH RESTRICTION ON SYNAPTOGENESIS IN THE PERINATAL GUINEA PIG HIPPOCAMPUS

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Objectives: Neonates born with a weight below the tenth percentile and asymmetric growth are identified as being growth restricted in fetal life due to sub-optimal *in utero* environment. Placental dysfunction and chronic hypoxia that manifest as fetal growth restriction (FGR) may lead to aberrant neuronal connectivity underlying risk for later neurologic disorders including cognitive deficits, attention deficit hyperactivity disorder and schizophrenia. Synapse formation occurs rapidly over the perinatal period in precocious brain developers, including humans and guinea pigs, and is critically dependent on nutrient supplies and activity-dependent remodelling. Synaptophysin (SYN) is a synaptic vesicle protein regulating synapse formation and maturation, and whose expression is proportional to the time course of synapse development. The purpose of this study was to determine the impact of FGR on synaptogenesis as measured by the immunoreactivity (IR) of SYN in the hippocampus of fetal guinea pigs near term, an area central to cognition and particularly sensitive to hypoxic insult. **Methods:** At mid-gestation (term ≈ 65 days) and under anaesthesia, pregnant guinea pigs were subjected to uterine artery ligation or uterine artery branch diathermy to induce FGR or sham surgery. Near term, the fetal pups were sacrificed, their organs extracted and weighed, and fetal brains were perfusion fixed and paraffin embedded for immunohistologic examination. SYN IR in the CA1, CA3 and dentate gyrus (DG) regions of the hippocampus was scored using Image Pro Plus 6.0 to quantify the percent area positively stained. Appropriate for gestational age (AGA, n=12, birth weight=88.8±0.8g (SEM), brain:liver=0.67±0.02), small for gestational age (SGA, n=8, birth weight=66.6±3.1g, brain:liver= 0.70±0.03) and FGR (n=8, birth weight=53.4±3.7g, brain:liver=1.13±0.08) pups were analyzed for SYN staining. **Results:** SYN IR % area staining for AGA animals measured 24 ± 3%, 18 ± 1%, and 19 ± 1% in the CA1, CA3, and DG regions respectively. This decreased in SGA animals at 13 ± 3%, 10 ± 2% (both $p < 0.02$) and 15 ± 1% (NS) and in FGR animals at 20 ± 2%, 15 ± 2% and 15± 2% (all NS). SYN staining is decreased by 15-20% in FGR animals and 15-44% in SGA animals compared to the AGA animals in the various regions of the hippocampus. **Conclusion:** The decrease in SYN in the hippocampus suggests growth restriction reduces SYN IR which may indicate a delay in the maturation of synapses or a reduction of the number of formed synapses both of which could impact neurological health and synaptic remodelling. Funding from Children's Health Research Institute and the Canadian Institute for Health Research.

VARIANTS OF RECEPTOR FOR ADVANCED GLYCATED END PRODUCTS IN HUMAN HEALTHY TERM PLACENTA

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Objectives: The receptor for advanced glycated end products (full-length RAGE, flRAGE) is a multiligand, membrane-anchored receptor with largely unknown physiologicalMS functions. Inflammatory states increase levels of ligands (AGEs, S100 proteins, HMGB1), followed by ligand-RAGE interaction, intracellular signaling MSand a cell response that promotes inflammation. A secreted soluble form, sRAGE produced by alternative splicing (RAGE_v1) or proteolytic cleavage, acts as a decoy to prevent interaction of RAGE with pro-inflammatory ligands. Most likely, sRAGE, flRAGE and ligands represent elements of a balance that is modified by pathophysiological situations. Preeclampsia (PE) is characterized by systemic inflammation, and increased levels of diverse RAGE-ligands in sera and placentas of women with PE have been demonstrated. RAGE protein expression has been shown previously in healthy placentas and there is MSevidence for increased RAGE expression in PE-affected placentas, suggesting involvement of the ligand-RAGE axis in the promotion of inflammation in PE. To gain an understanding of MSplacental RAGE functions in health and disease, our aim was to determine the major RAGE variant(s) expressed in healthy human placenta. **Methods** : Placental chorionic tissue of patients that underwent elective Caesarean MS sections at term was studied. To obtain isolated trophoblast cells, enzymatic dissociation of term villous placental tissue, followed by Percoll gradient separation and in vitro culture (72h) was performedMS. RT-PCR and real-time RT-PCR were used to investigate RAGE mRNA variants. Four anti-RAGE antibodies (Abs) directed against either the whole molecule (1), the extracellular region (2,3) or the cytoplasmic domain (4) were employed to study RAGE protein expression by western blotting. Abs 2 and 3 were used to localize RAGE in placental chorionic tissue by indirect immunofluorescence (IF) microscopy. **Results:** flRAGE and RAGE_v1 are the major mRNA species in total placental tissue as well as in isolated and in vitro differentiated trophoblast cells, with a greater abundance of flRAGE. In western blotting experiments, Abs against the whole molecule or cytoplasmic domain detected a protein of ~50-55 kDa. Abs against the ectodomain detected either two bandsMS of comparableMS intensity (Ab2; ~50-55 kD, ~45-50 kDa), or mainly the smaller protein (Ab3). sRAGE and flRAGE have a known size of ~48 kDa and ~55 kDa, respectively, therefore we conclude that both flRAGE and sRAGE protein are expressed in healthy term placental tissue. Indirect IF microscopy detected RAGE variants in the syncytiotrophoblast, but also in cells in the stroma of the villi including endothelial cells. **Conclusion:** Human term chorionic tissue expresses flRAGE and sRAGE at mRNA and protein level. As both RAGE variants are present in the syncytiotrophoblast in direct contact with maternal blood, an involvement of the RAGE system in inflammatory processes during pregnancy can be assumed.

INTERACTIVE EFFECTS ALCOHOL AND ESTROGEN ON PREGNANT UTERINE ARTERY ENDOTHELIAL CELL PROLIFERATION

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Background: A cardinal feature of Fetal Alcohol Spectrum Disorders (FASD) is Intrauterine Growth Restriction (IUGR), and models of IUGR demonstrate dysfunctional maternal uterine vascular adaptations including lower uterine blood flow and decreased angiogenesis. This altered remodeling is mediated in part by estrogen, which is significantly elevated during gestation. Further, estrogen specifically induces ovine pregnant uterine artery endothelial cell (P-UAEC) proliferation. **Hypothesis:** In this study, we hypothesized that there exists an interaction between estrogen and alcohol on ovine P-UAEC proliferation. **Methods:** P-UAECs were isolated from pregnant ewes (gestational day 120-130; term = 147), FACS sorted, validated, and maintained in culture to passage 4. To mimic maternal binge drinking patterns, P-UAECs were cultured in the absence or presence of alcohol (150 mg/dl) in a compensating system for 3 h on 3 consecutive days. Cell viability was validated by trypan blue exclusion microscopy, Calcein AM imaging, and immunoblotting. Following this treatment paradigm, control and binge alcohol treated P-UAECs were abstained from alcohol for two days and then treated with increasing concentrations of estrogen (0.1, 1, 10 nM) in the presence or absence of alcohol. Angiogenesis index was evaluated using BrdU Proliferation Assay. **Results:** Trypan blue stained cell count demonstrated that the number of viable cells in the control and binge alcohol groups were not different (control, 891,250 ± 11,433; alcohol, 834,000 ± 49,784; P = 0.73). We also observed that uncleaved caspase 3 was unaltered by binge alcohol and cleaved caspase 3 was not detectable demonstrating cell viability. A significant main effect of the alcohol treatment paradigm was noted (two way ANOVA, P = 0.003). Irrespective of the dose, estrogen-induced P-UAEC proliferation was significantly decreased in response to chronic binge alcohol (P = 0.025). In contrast, estrogen-induced proliferation trended higher in response to acute alcohol treatment (P = 0.057). Further, estrogen-induced proliferation in chronic binge treated P-UAECs was significantly lower compared to acute alcohol treatment (P <0.001). Interestingly, these findings were closely associated with the effects of binge alcohol on the activity state and expression of the angiogenesis-associated enzyme eNOS and its related signaling cascades. Alcohol decreased P^{635}eNOS, total eNOS, PERK1, total ERK1, and total ERK2 to 15%, 43%, 24%, 16%, and 21%, respectively whereas no alterations were observed with the AKT pathway. **Conclusions:** Thus: 1) proliferative effects of alcohol depend on the alcohol treatment paradigm; 2) chronic binge alcohol impairs estrogen-induced proliferation; 3) binge alcohol decreases the expression and activity of eNOS, a protein associated with P-UAEC proliferation; 3) effects of chronic binge alcohol on P-UAECs are specifically associated with ERK cascade not AKT pathway; and 5) these findings support the idea that the uterine compartment may play a key role in the pathogenesis of FASD. NIH AA19446, HL49210, HL87144 and HD38843

THE ROLE OF MATERNAL OBESITY ON THE SYMPATHETIC NERVOUS SYSTEM IN OFFSPRING

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Objectives: The prevalence of obesity continues to increase in the general population in the UK and throughout the world. Consequently maternal obesity rates are rising, placing both mother and fetus at risk of adverse outcomes. Current evidence suggests that there could be a link between maternal BMI, hypertension and sympathetic activation. In this study we examined the effects of maternal obesity and weaning environment on male mice through measuring the effects on heart rate variability, body mass, organ weight and renal norepinhephrine levels. **Methods:** Female C57BL/6J mice were either fed a semi-synthetic, highly palatable high fat obesogenic high fat diet (10% simple sugars, 20% animal lard, 28% polysaccharide, 23% protein [w/w], Special Dietary Services, energy 4.5cal/g) or a control standard chow diet (7% simple sugars, 3% fat, 50% polysaccharide, 15% protein [w/w] RM1, Special Dietary Services, energy 3.5kcal/g) for six weeks prior to mating, throughout mating, pregnancy and the lactation period. During the weaning period, the male offspring from the control diet were divided into two groups and randomized so that half remained on the control diet (OffC-C) and half went onto the obesogenic diet (OffC-Ob). Half of the male offspring who received the obesogenic diet throughout gestation remained on the obesogenic diet (OffOb-Ob) and the other half was transferred onto the control diet (OffOb-C). Radiotelemetry devices were implanted to measure the high and low frequency (LF) bands of heart rate variability (HRV) as indices of autonomic activity. At 90 days the mice were sacrificed; their body mass and various organ weights were weighed and then cryopreserved. The concentration of norepinephrine in the left kidney of male and female mice was later measured by ELISA. **Results:** There was a statistically significant increase in the total body weight and increase in various organ weights of all offspring receiving the obesogenic diet either during gestation or weaning. Predictably the highest body mass ($p<0.001$) and organ weights were seen in the offspring who received an obesogenic diet throughout gestation and weaning periods, with an increase in all organ weights measured ($p<0.05$) but not muscle mass compared to the control. There was a decrease in the mean low frequency activity in mice weaned to an obesogenic diet ($p<0.01$) but no other significant effects on HRV. Male OffOb-C mice and female OffC-Ob and OffOb-C mice had raised renal norepinephrine levels ($p<0.01$, 0.01, 0.05 respectively). **Conclusion:** An obesogenic diet leads to raised body mass and organ weights and increased renal norepinephrine levels. However, contrary to the expected results there was no additive effect of an obesogenic weaning diet on HRV, suggesting minor influence on sympathetic drive after weaning.

THE ROLE OF LEPTIN ON THE SYMPATHETIC NERVOUS SYSTEM IN NEONATAL RATS

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Objectives: Leptin has a well known role in modulating normal appetite and energy balance. However, recently leptin resistance manifest by alterations in leptin receptor and anatomical pathways have been shown to be associated with obesity. This effect of leptin in obesity could be mediated through its activation of the sympathetic nervous system. The aim of this study was to investigate the role of leptin neonatally and its effects on heart rate, heart rate variability, blood pressure, renal norepinephrine concentration, body weight and organ weights. **Methods:** Neonatal male and female Sprague-Dawley rats were given either 3μ/kg of saline to control rats or 3μ/kg of leptin intraperitoneally twice a day between postnatal day 8 to 14. The heart rate variability (HRV), day and night time systolic blood pressure and systolic blood pressure and heart rate response to restraint stress were measured through an implanted PA-C10 transmitter radiotelemetry device (Data Science International Inc [DSI] PhysioTel mouse PA-C10; DSI, St. Paul, MN, USA). The rats were then sacrificed at either 30 or 60 days and their body weight abdominal and subcutaneous white adipose tissue, brain, heart, liver, left and right kidneys, pancreas, adrenal, and brown adipose tissue weights were recorded and then cryopreserved. Subsequently, the level of norepinephrine in the left kidney was measured by ELISA. **Results:** At 30 days male leptin treated rats had raised heart weight ($p<0.05$) and female leptin treated rats had increased abdominal white adipose tissue ($p<0.05$), heart weight ($p<0.01$) and pancreas weight ($p<0.01$) compared to the saline treated controls. Male and female leptin treated rats had an increased abdominal white adipose tissue ($p<0.05$, 0.01), heart weight ($p<0.05$) and pancreas weight ($p<0.05$) compared to the controls by 60 days. The average night time systolic blood pressure was raised in the leptin treated animals ($p<0.01$). Gender differences were observed in response to stress restraint with the female leptin treated group having raised heart rate and systolic blood pressure in response to stress ($p<0.01$) and longer time to return to baseline levels ($p<0.05$). In regard to HRV, the only statistically significant effect was an increase in the Low/High frequency band ratio, an indicator of increased sympathetic activity, in females ($p<0.01$). Leptin increased the renal norepinephrine levels in both males and females ($p<0.05$). **Conclusion:** Supplemental leptin treatment in rats was associated with increased body mass, increased renal norepinephrine concentration, and night time hypertension. However, abnormal stress related adaption and HRV to restraint stress was only observed in females, suggesting gender-specific modification of sympathetic activity.

STRUCTURAL ABNORMALITIES INDUCED BY CHRONIC HYPOXIA IN UTERO LEAD TO VASCULAR DYSFUNCTION IN ADULTHOOD

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Objectives: In developed countries chronic hypoxia due to placental insufficiency accounts for the majority of intrauterine growth restriction (IUGR). Mechanisms underlying the susceptibility to cardiovascular disease (CVD) in adults who were growth restricted in the womb remain unknown. With the use of an ovine model replicating the human condition, we previously found chronic hypoxia to alter aortic and cardiac remodeling in the fetus. This hypoxic-induced effect included media hypertrophy, intima hyperplasia and altered composition of the extracellular matrix proteins that are critical in the buffering function of the aorta. A second animal model was applied to determine if these structural abnormalities persist into adulthood and manifest in vascular dysfunction. **Methods:** Pregnant guinea pigs underwent uterine artery ligation at mid-gestation in order to induce chronic fetal hypoxemia. One group of animals was sacrificed at term and a second group was allowed to pup and sacrificed in adulthood. Vessels harvested from both groups were analyzed for collagen and elastic fibre content using histological procedures. Mesenteric arteries and the descending aorta were excised from adult animals and immediately placed in physiological saline. Subsequently, vascular responses were assessed using a pressure myograph for mesenteric arteries and a vessel bath apparatus for aortic rings. Length-tension curves were measured in additional aortic rings. **Results:** In both groups, ligated guinea pigs were growth restricted as revealed by term/birth weights ($p < .05$). The brain:liver ratio in IUGR term fetuses was greater compared to control fetuses ($p < .05$). Structural abnormalities associated with chronic hypoxia as revealed by our ovine model, were present in ligated fetuses and adults. Aortae from IUGR offspring exhibited blunted responses to a nitric oxide (NO) donor and exaggerated responses to Angiotensin (ANG) II ($p < .05$). Vasoconstrictor and vasodilator responses of the mesenteric artery were not different between IUGR and control offspring. The length tension curve of aortic rings cut from IUGR offspring were shifted to the left in comparison to control ($p < .001$). **Conclusion:** Central arterial stiffness is a strong and independent predictor of cardiovascular disease. Increased aortic stiffness present in IUGR offspring is traced to hypoxic-induced structural changes established in fetal life. IUGR offspring also exhibit additional functional abnormalities present in hypertensive patients, including disturbed vascular responses to NO and ANG II. This reduced effectiveness of NO to cause vasodilation may be due to oxidative stress associated with advanced atherosclerotic lesions. Thus perturbations in vascular remodeling appear to underlie programming of cardiovascular disease in the case of placental insufficiency.

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HIGH ALTITUDE, NATURAL SELECTION, & PREGNANCY: IS BIRTH WEIGHT PROTECTED IN THE ANDES?

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Objectives: Fetal growth is slowed at high altitude (HA) and preeclampsia more common. Populations of multi-generational residence at HA show a decrease in fetal growth at high altitude, but the decrease is less than that seen in relative newcomers to HA. There is debate as to whether decreased birth weight at HA has been selected for or against. We explored the relationship between genes that show evidence of natural selection and their effect on phenotype by 1) identifying hypoxia-related gene regions that showed evidence of natural selection through the analysis of genome-wide SNP microarray data (Bigham et al., 2009), and 2) searching for association between genotypes at candidate loci, circulating gene product (protein) levels, uterine artery (UA) blood flow and other pregnancy phenotypes at HA. **Methods:** Maternal and fetal phenotypes through pregnancy were measured serially in 52 native Andeans living at 3800m at weeks 23, 31, and 37 of pregnancy and 3 months postpartum. Genotypes were measured using the Affymetrix 500K Array (Bigham et al. 2009) and RT-PCR and protein was measured using enzyme-linked immunosorbent assays. **Results:** In 55 multi-generational HA pregnant Andeans, the epithelial cadherin (CDH1) gene region SNP genotypes were associated with UA blood flow. Soluble CDH1 (seCAD) correlated with CDH1 genotype and also with UA blood flow. Additionally, AMP-activated kinase, alpha-1 subunit (AMPKa1) genotype was strongly associated with gestational age at birth and aryl-hydrocarbon receptor nuclear translocator 2 (ARNT2) was associated with birth weight. **Conclusions:** In all cases, alleles more frequently found in Andeans were positively associated with UA blood flow, gestational age at birth, or birth weight, suggesting evolution at HA may have selected for increased birth weight, as opposed to "small-but-healthy" babies, in adapted populations. We thank the National Institutes of Health (HL-60131, TN-01188, and HL-07171) National Science Foundation (Graduate Research Fellowship) and American Heart Association (Predoctoral Fellowship 0610129Z) for financial support.

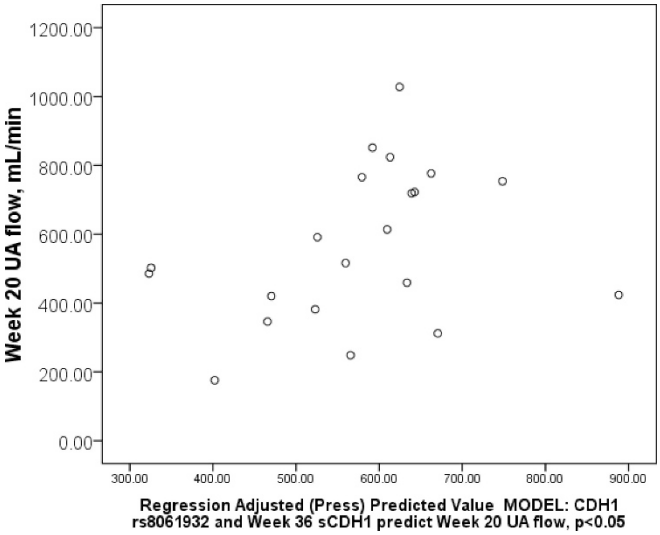

Figure. CDH1 rs8061932 genotype and sCDH1 at week 36 predict UA flow at week 20 (model $p<0.05$, linear regression).

ADRENAL DEMEDULLATION ABOLISHES HYPOXEMIA-INDUCED CATECHOLAMINE SUPPRESSION OF GLUCOSE STIMULATED INSULIN SECRETION IN FETAL SHEEP

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Hypoxemia stimulates catecholamine secretion from adrenal chromaffin cells, which then suppresses insulin secretion in fetal sheep. Previous studies show that norepinephrine (NE) is the predominant catecholamine released, but that adrenal demedullation (AD) does not completely eliminate plasma NE. Our objective was to abolish hypoxemia-induced catecholamine secretion via bilateral AD and determine whether this restores glucose stimulated insulin secretion (GSIS) during fetal hypoxemia. At 123 days of gestation (term 147 days), surgical AD was carried out on six fetuses and their responsiveness compared to sham operated controls ($n = 2$). At autopsy, completeness of AD was confirmed in four fetuses via immunostaining for chromogranin A, while two fetuses with chromaffin tissue remaining were included with sham controls ($n = 4$). Two square-wave hyperglycemic clamps were performed at 128 days to measure GSIS during normoxemia (24.7 $\pm$ 0.8 mmHg O2) or hypoxemia (14.1 $\pm$ 0.5 mmHg O2), induced by bleeding nitrogen gas ($\sim$ 6 L/min) into a maternal trachea catheter. Steady state plasma glucose at baseline did not differ ($P > 0.454$) between controls and AD fetuses during normoxemic (1.19 vs. 1.26 $\pm$ 0.06 mmol/L, respectively) or hypoxemic (1.19 vs. 1.09 $\pm$ 0.06 mmol/L) GSIS studies. Likewise, baseline plasma insulin was not different ($P > 0.463$) between treatments during normoxemia (0.38 vs 0.48 $\pm$ 0.06 ng/mL) or hypoxemia (0.23 vs. 0.28 $\pm$ 0.06 ng/mL). Plasma NE concentrations were similar ($P = 0.248$) in control and AD fetuses (544 vs. 444 $\pm$ 84.8 pg/mL, respectively) during normoxemia, but were 3.3-fold greater ($P = 0.034$) in control fetuses during hypoxemia. Also, plasma insulin concentrations during hypoxemia were higher ($P = 0.021$) in response to hyperglycemia (2.62 $\pm$ 0.24 mmol/L) in AD compared to controls (0.73 vs. 0.22 $\pm$ 0.14 ng/mL, respectively) and were, in fact, comparable to normoxemic control levels (0.93 $\pm$ 0.14 ng/mL), demonstrating an inverse relationship between NE and insulin ($r = -0.22$; $P < 0.05$). Plasma epinephrine concentrations were not increased during hypoxemia and were not different ($P = 0.422$) between treatments. These findings indicate that the adrenal medulla is the primary site for acute hypoxia-stimulated NE secretion in fetal sheep at 128 days, as plasma NE concentrations were unchanged by hypoxemia in sheep fetuses with complete surgical ablation of the adrenal medulla. Furthermore, elevated NE diminishes GSIS and AD rescues GSIS, showing hypoxemia acts indirectly through NE. Interestingly, no effects of AD were observed in normoxic conditions, which indicate marked quiescence of the adrenal chromaffin cells in a favorable intrauterine environment. Additionally, NE was not affected by glycemic levels under normoxemic or hypoxemic conditions. In conclusion, elevated NE suppresses GSIS during late gestation in fetal sheep, but surgical ablation of the adrenal medulla restores this insulin secretion responsiveness.