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MEETING ABSTRACTS

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THE EFFECT OF ADOLESCENCE ON FETAL, PLACENTAL, AND UTERINE DEVELOPMENT, PLACENTAL VASCULAR MEASURES, AND THE CORRELATION OF CHANGES IN PLACENTAL VASCULARITY WITH EXPRESSION OF MAJOR ANGIOGENIC FACTORS AND THEIR RECEPTORS IN SHEEP

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Objectives: We proposed that the effect of maternal age on placental development can be explained by reduced placental vascular development and that there is correlation between vascularity changes and the expression of the major angiogenic factors and/or their receptors in the placenta. We determined the effect of maternal age on maternal placental (caruncle; **CAR**), fetal placental (cotyledon; **COT**), total placental and fetal membrane weights, and also evaluated CAR and COT vascularity and mRNA expression for angiogenic factors in these tissues. **Methods:** Straight-bred singleton pregnancies were established by embryo transfer in 13 Columbia (**C**) recipients (6 peri-pubertal lambs, **PP;** and 7 yearling, early adults, **EA**) and 13 Romanov (**R**) recipients (7 PP and 6 EA). Uteri were collected on day 135 of gestation, and fetuses, fetal membranes and CAR and COT samples were weighed. Placentomes were fixed with Carnoy's solution by perfusion of the arterial vessels supplying the CAR or COT, then embedded in paraffin, sectioned, and stained with hematoxylin and periodic acid-Shiff's. Photomicrographs were taken and vascularity was determined by image analysis (Image-Pro Plus). For CAR and COT we determined: the cross-sectional capillary area density (**CAD**), capillary number density (**CND**), capillary surface density (**CSD**), and average cross-sectional area per capillary (**APC**). Samples of CAR and COT were snap frozen and later analyzed for vascular endothelial growth factor (*VEGF*), VEGF receptor-1 and -2 (*FLT1,* and *KDR*), placental growth factor (*PlGF*), neuropilin-1 and -2 (*NP1,* and *NP2*), angiopoietin-1 and -2 (*ANGPT1* and *2*), receptor for both ANGPT (*TEK*), basic fibroblast growth factor (*FGF2*), endothelial nitric oxide synthase (*NOS3*), soluble guanylate cyclase (*GUCY1B3*), and hypoxia inducible factor-1 (*HIF1A*) mRNA expression by quantitative, real-time RT-PCR for PP and EA. **Results:** For both breeds, fetal weight and fetal membrane weight were less ($P < 0.04$; 2,977 vs. 3,523 g for R, SE = 0.18; 5,258 vs. 6,228 g for C, SE = 319; and $P < 0.09$; 197 vs. 256 g for R, SE = 13.9; and 290 vs. 338 g for C, SE = 9.81, respectively). For CAR of both breeds, CAD was reduced ($P < 0.04$) in PP vs. EA (0.35 vs. 0.41, SE = 0.04). Within the breed, CSD in C CAR and CND in R COT tended to be reduced ($P < 0.08-0.09$) in PP vs. EA. Vascularity changes in R COT were correlated with *FLT1* mRNA expression in PP and EA ($R^2 = 0.83$, $P < 0.04$; $R^2 = 0.84$, $P < 0.04$, respectively) and *VEGF* mRNA in PP ($R^2 = 0.73$; $P < 0.09$) although the mRNA expression did not differ. In COT of R and C, *ANGPT2, HIF1A,* and *TEK* mRNA expression were less in PP vs. EA ($P < 0.04$ –, 0.07). **Conclusions:** These data indicate that maternal age affects placental vascular development and expression of mRNA for major angiogenic factors. Supp. by *NIH grant HL64141 to LPR and DAR; and NIH grant P20 RR016741 from INBRE.*

GLUCOSE HOMEOSTASIS IN SHEEP DURING LATE PREGNANCY AND EARLY LACTATION IS AFFECTED BY THE LEVEL OF NUTRITION RECEIVED DURING LATE FETAL LIFE

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Objectives: In the present study it was investigated whether adaptation of glucose homeostasis and metabolism to pregnancy and lactation in sheep is dependent on the level of nutrition received during late fetal life. **Methods:** 24 ewes, born to sheep offered either a NORM (~15 MJ ME/d) or a LOW diet (~7 MJ ME/d) during the last six weeks pre partum, were used. The experimental ewes at approximately one years of age were subjected to intravenous glucose tolerance tests (IGTT) in late gestation: one prior to (G-IGTT), another by the end of a five day feed restriction period (50%; RG-IGTT), and a third challenge test around expected peak lactation (L-IGTT). **Results:** Ewes exposed to a LOW level of nutrition during late fetal life had lower insulin concentrations during lactation and a decreased absolute insulin secretion compared to the NORM ewes, although glucose tolerance during the L-IGTT was similar in the two groups. This indicates increased insulin sensitivity during lactation in LOW ewes. During late gestation (G-IGTT and RG-IGTT) glucose stimulated insulin secretion was depressed compared to the L-IGTT in both groups of ewes. Glucose tolerance was of similar magnitude in the two groups during G-IGTT in the fed state. However, in response to a feed restriction during late gestation, LOW became more glucose intolerant and apparently more insulin resistant compared to the ewes exposed to a NORM nutritional level during late fetal life. **Conclusions:** Undernutrition during late fetal life impairs the pancreatic insulin secretory capacity in the adult ewe, and reduces plasticity of down regulation of insulin secretion in response to a metabolic challenge (late gestation and feed restriction). Furthermore late fetal life undernutrition appears to induce encoding of mechanisms, which can change the peripheral insulin hyper sensitivity observed during the relatively non-challenging early lactation situation into an insulin resistance observed in the young adults during late gestation and feed restriction.

EFFECT OF MATERNAL NUTRITION FROM MID-PREGNANCY ON THE SUMMIT METABOLIC RATE OF TWIN-BORN LAMBS

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Objectives: Maternal nutrition during gestation can influence fetal development and may affect the ability of the newborn lamb to thermoregulate after birth. The influence of maternal nutrition from day 70 of gestation on the summit metabolic rate in twin-born lamb at 24 to 36 hours of age was investigated. **Methods:** Twin-bearing ewes were fed at below (underfed; n = 17) or above (well fed; n = 17) maintenance by offering different pasture levels from pregnancy day 70 until 24 hours after parturition. At pregnancy day 100, half of the ewes from each nutritional treatment were offered 400 g/ewe/day of concentrate supplement until parturition. At 24 to 36 hours after parturition lambs were removed from their dams and their summit metabolic rate was measured using indirect calorimetry. **Results:** Ewe nutritional treatment had a significant effect on ewe liveweight gain from pregnancy day 70 until parturition (underfed; 10.5 ± 1.35 kg; underfed plus supplement, 14.3 ± 1.28 kg; well fed, 24.3 ± 1.28 kg; well fed plus supplement, 27.9 ± 1.35 kg; $p < 0.001$). Lambs born to ewes that were well fed with supplement had significantly heavier birth weights than lambs born to ewes on all other nutritional treatments (well fed plus supplement, 5.6 ± 0.16 kg; well fed, 4.9 ± 0.16 kg; underfed plus supplement, 4.8 ± 0.17 kg; underfed, 4.8 ± 0.15 kg; $p < 0.05$). Lamb summit metabolic rate did not differ between treatment groups, but lambs born to underfed ewes and well fed plus supplement ewes took a significantly longer period of time to reach summit metabolic rate than lambs born to ewes offered other nutritional treatments (underfed, 56.8 ± 5.79 min; underfed plus supplement, 31.2 ± 6.23 min; well fed, 40.4 ± 5.56 min; well fed plus supplement, 49.9 ± 6.21 min; $p < 0.01$). **Conclusions:** Different maternal nutrition from mid pregnancy to parturition has no impact on lamb summit metabolic rate at 24 to 36 hours of age. It does however have an impact on the time the lamb took to reach summit metabolic rate. Lambs born to ewes offered the two extremes of nutrition, underfed or well-fed with supplement, took longer to reach summit metabolic rate. Lambs born to ewes that were well fed with supplement were significantly heavier than lambs born to other ewes. The heavier lamb will have a smaller surface area: body weight ratio compared to lighter lambs and therefore may lose less heat to the environment. This may explain why they took longer to reach summit metabolic rate. The fact that lambs born to well-fed ewes with supplement and underfed ewes took longer to reach summit metabolic rate also supports previous work that additional or restricted maternal nutrients during gestation may "program" and cause the fetus to thermoregulate more efficiently as a neonate. The difference in time to reach summit metabolic rate suggests that there is an optimal energy requirement for adipose tissue development and temperature regulation after birth.

EFFECT OF BIRTH RANK ON THE PHYSIOLOGICAL STATUS OF THE NEWBORN LAMB

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Objectives: As fetal number increases, so does the competition between fetuses for the limited supply of maternal nutrients. Nutrient availability during gestation will differ in single-(s), twin-(tw) and triplet-born (tr) lambs. The influence of lamb birth rank on the physiological status of the newborn lamb from birth to 24 hours of age was investigated. **Methods:** Single, twin and triplet-bearing ewes were chosen at pregnancy day 50 and were offered *ad-libitum* pasture during pregnancy until parturition. Lamb blood samples were taken at 0, 3, 12 and 24 hours post-birth and rectal temperature was taken at 0, 1, 3, 6 and 12 hours post-birth. Lamb body weight, crown-rump length and girth circumference were measured at 3 hours of age. **Results:** Triplet-born lambs had shorter gestation lengths than single- or twin-born lambs (s, 146.3 ± 0.43 days; tw, 146.0 ± 0.22 days; tr, 145.0 ± 0.20 days; $p < 0.001$). Single- and twin-born lambs were heavier (s, 5.7 ± 0.20 kg; tw, 5.0 ± 0.10 kg; tr, 4.0 ± 0.09 kg; $p < 0.0001$), longer (s, 54.9 ± 1.51 cm; tw, 51.8 ± 0.77 cm; tr, 48.7 ± 0.73 cm; $p < 0.001$) and had greater circumference (s, 41.5 ± 0.70 cm; tw, 40.5 ± 0.36 cm; tr, 37.0 ± 0.33 cm; $p < 0.0001$) than triplet-born lambs. Directly after birth, single- and twin-born lambs had greater plasma triiodothyronine (T3) concentrations than triplet-born lambs (s, 3.5 ± 0.30 nmol/L; tw, 2.9 ± 0.16 nmol/L; tr, 2.3 ± 0.14 nmol/L; $p < 0.001$). Single-born lambs had greater plasma thyroxine (T4) concentrations than both twin- and triplet-born lambs (s, 172.8 ± 12.0 nmol/L; tw, 143.5 ± 6.27 nmol/L; tr, 129.6 ± 5.72 nmol/L; $p < 0.001$). In addition, all birth ranks had different log transformed plasma fructose concentrations (s, 1.1 ± 0.12 mmol/L; tw, 0.9 ± 0.05 mmol/L; tr, 0.6 ± 0.06 mmol/L; $p < 0.001$). Lactate, packed cell volume and cortisol plasma concentrations did not differ between birth ranks. The mean temperature from birth to 24 hours of age triplet-born lamb rectal temperature was lower than single or twin born lamb rectal temperature (s, 40.0 ± 0.16°C; tw, 39.7 ± 0.08°C; tr, 39.5 ± 0.07°C; $p < 0.05$). Log transformed plasma glucose concentrations were different for all birth ranks (s, 6.7 ± 0.33 mmol/L; tw, 4.5 ± 0.17 mmol/L; tr, 3.8 ± 0.16 mmol/L; $p < 0.0001$). **Conclusions:** Birth rank has a significant effect on the physiological status of the newborn lamb. Triplet-born lambs have a shorter gestation length, lower birth weight, lower plasma concentrations of glucose, fructose and thyroid hormones (T3, T4) and lower rectal temperatures within the first 24 hours of life. These postnatal factors all suggest that the triplet-born lamb are less developed than their single- and twin-born counterparts. This may be due to competition with other fetuses during gestation for nutrient supply. This competition and nutrient restriction may have a negative impact on individual fetal growth, development and postnatal survival.

VIAGRA (SILDENAFIL CITRATE) TREATMENT ENHANCED FETAL GROWTH IN AN OVINE MODEL OF INTRAUTERINE GROWTH RETARDATION

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Objectives: Determine the effects of Viagra on fetal and placental growth in an ovine model of intrauterine growth retardation (IUGR). Intrauterine growth retardation (IUGR) is a common pregnancy complication in a variety of species, including humans, sheep, pigs, cows, horses, and rats. IUGR can result from insufficient nutrient partitioning to the fetus due to maternal undernutrition or perturbations in uterine blood flow and placental angiogenesis. This study determined the effects of Viagra on fetal and placental growth in an ovine model of IUGR. **Methods:** Sixty Suffolk ewes were randomly assigned to one of six treatments in a 2 × 3 factorial study. Ewes received either 100% (adequately fed) or 50% (underfed) NRC requirements from gestational days 28 to 112 based on body weight assessed weekly. Within each diet, ewes received i.m. injections of either 0, 25, or 50 mg Viagra at 0700, 1500, and 2300 h daily. Tissue and blood samples were collected on day 112. **Results:** Almost every parameter measured in the dam and fetus was affected by litter size; therefore, only data from singleton pregnancies will be presented. Maternal body weight and body condition score (BCS) were decreased ($P < 0.001$) by diet but not Viagra treatment in underfed ewes. Maternal gastrocnemius muscle ($P < 0.01$), intestine ($P = 0.07$), kidney ($P = 0.07$), liver ($P < 0.001$) weights, and maternal plasma levels of total amino acids ($P < 0.01$) were decreased in underfed ewes. Maternal heart ($P < 0.05$) and pancreas ($P = 0.07$) weights were affected by the interaction of diet and dose, in that the 75 mg dose of Viagra decreased ($P = 0.07$) heart weight in underfed ewes, whereas the 150 mg dose of Viagra decreased ($P = 0.07$) heart weight but increased ($P = 0.06$) pancreas weight in adequately fed ewes. Uteroplacental weight was greater ($P = 0.02$) in adequately fed than in underfed ewes (11.9 vs 10.9 lbs, SE = 0.3) and was not affected by Viagra dose or their interaction. Placentome weight, placentome number, or volume of amniotic and allantoic fluids were not affected by diet or Viagra dose. Fetal weight and fetal plasma levels of total amino acids were reduced ($P < 0.05$) by underfeeding, but were increased ($P = 0.08$) by the Viagra treatment. Underfeeding also decreased ($P < 0.05$) fetal liver, pancreas, and kidney weights, whereas Viagra increased ($P < 0.05$) the weights of fetal spleen as well as septum, left ventricle and right ventricle of the heart. Viagra at the 150 mg dose increased fetal weight by 17% and 23% in adequately-fed and underfed ewes, respectively. **Conclusions:** Results of this study indicate that Viagra administration may be an effective tool to increase fetal growth in both underfed and adequately nourished ewes. (Supported by a Pfizer Viagra Grant No. 594).

NUTRITIONALLY-MEDIATED PRENATAL GROWTH RESTRICTION AND POSTNATAL HYPOTHALAMIC-PITUITARY-ADRENAL FUNCTION IN FEMALE SHEEP

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Adolescent girls who continue to grow while pregnant have a high risk of prematurely delivering low birth weight infants. Similarly, when pregnant adolescent sheep are overnourished to promote rapid maternal growth during pregnancy, growth of the placenta is impaired, limiting absolute fetal nutrient supply. By late pregnancy these fetuses are ~30% smaller than normally growing controls (N) but have higher brain, perirenal fat and adrenal gland mass per kg fetus; they are also delivered ~3 days earlier than the N group, which may be an indicator of premature activation of the fetal hypothalamic-pituitary-adrenal (HPA) axis.

Objective: To examine the ontogeny of HPA responses in low and N birth weight female offspring at 3 postnatal ages. **Methods:** Adolescent dams were offered high or control nutrient intakes to induce low (L, n = 10) or normal (N, n = 8) birth weight, respectively (3279 vs. 5498 g, $P < 0.001$). Thereafter, dams were fed to maximise milk yield, and following weaning, lambs were fed *ad libitum*. At 9, 18 and 24 months of age, the ACTH and cortisol responses to corticotrophin releasing hormone (CRH) plus arginine vasopressin (AVP) were measured. **Results:** Fractional growth rate to weaning at 3 months was higher in L vs. N offspring (10.7 vs. 6.5%/day, $P < 0.0005$) reflecting rapid postnatal catch up growth in low birth weight females. At 3, 9, 18 and 24 months, body weight and adiposity scores were independent of birth weight and prenatal diet, and all animals became progressively obese with age. At necropsy at 24.5 months, internal fat mass was equivalent between groups but absolute and body weight specific mass of the hocks was reduced ($P < 0.01$) in the L group indicating that adult height was permanently impaired by prenatal growth restriction. Total kidney nephron number was lower in L vs. N groups ($P < 0.06$) and correlated with birth weight ($r = 0.51$, $P < 0.05$). Baseline ACTH and cortisol concentrations were independent of prenatal growth and, cortisol, but not ACTH, decreased with increasing age ($P < 0.001$). Prenatal growth restriction did not influence the ACTH or cortisol response to CRH/AVP at any stage studied. Area under the curve (AUC) and peak concentrations for ACTH increased linearly with postnatal age ($P < 0.001$) and at 24 months were on average two fold higher than at 9 months. In contrast the AUC for cortisol was equivalent at 9 and 24 and lower ($P < 0.001$) than at 18 months. **Conclusion:** Nutritionally-mediated prenatal growth restriction in females lambs is associated with rapid postnatal catch up growth with respect to weight and a permanently negative effect on stature. It is unlikely that this form of growth restriction influenced the maturation or programming of the fetal HPA as we failed to detect altered responsiveness at the postnatal ages studied. The mechanism underlying the increase in pituitary (ACTH) responsiveness with increasing age in *ad libitum* fed offspring is unknown but is commensurate with the parallel reduction in baseline cortisol and hence reduced negative feedback as the animals age.

AMP-ACTIVATED PROTEIN KINASE (AMPK) MAY BE RESPONSIBLE FOR THE DOWN-REGULATION OF INSULIN/IGF-1 SIGNALING IN COTYLEDONARY ARTERIES OF OBESE PREGNANT EWES

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Objectives: Estimates suggest that 18–35% of pregnant women in the USA are clinically obese. We investigated the impacts of maternal obesity in the ewe, on placental vascularity, a key determinant of fetal nutrient delivery. In the ewe, placentomes, sites of maternal:fetal nutrient exchange, are composed of maternal (caruncular) and placental (cotyledonary, COT) components. We previously reported that maternal obesity led to reduced vascularity in COT tissues of ewes by mid-gestation. Further, COT arteries exhibited decreased protein kinase B (Akt) and extracellular signal-regulated kinase 1/2 (ERK 1/2), signaling pathways known to facilitate angiogenesis. While the mechanism for decreased COT arterial Akt and ERK1/2 was unknown, we hypothesized that AMPK might be involved. Both Akt and ERK1/2 are down-stream components of insulin/IGF-1 signaling pathway, and AMPK is known to sensitize insulin/IGF-1 signaling through phosphorylation of insulin receptor substrate-1 (IRS-1). This study assessed the impact of maternal obesity on COT artery AMPK activity, and its role in down-regulation of insulin/IGF-1 signaling. **Methods:** Ewes were assigned to a control (C, 100% of NRC recommendations, n = 10) or obesogenic (OB, 150% of NRC, n = 10) diet from 60 days before to 75 days after conception when ewes were euthanized. At necropsy, the smallest terminal arteries entering COT tissues (0.5~1.0 mm in diameter) were collected and frozen in liquid nitrogen until protein extraction and western blotting. **Results:** At necropsy, fetal blood levels of glucose, insulin and IGF-1 were higher ($P < 0.05$) in OB than C ewes (43.63 ± 5.58 mg/dl, 7.29 ± 1.31 IU/ml, and 53.30 ± 1.76 ng/ml versus 25.35 ± 2.10 mg/dl, 1.14 ± 0.35 IU/ml and 44.62 ± 0.90 ng/ml, respectively). Total AMPK and AMPK phosphorylated at Thr 172 (the active form) were down-regulated ($P < 0.05$) by 19.7 ± 8.4% and 25.9 ± 7.7%, respectively, in the COT arteries of OB versus C ewes. Total acetyl-CoA carboxylase (ACC), a down-stream target of AMPK, and its phosphorylated form was also reduced ($P < 0.05$) by 32.9 ± 9.2% and 45.4 ± 14.6%, respectively, in OB compared to C COT arteries. The phosphorylation of IRS-1 at Ser789, a site phosphorylated by AMPK, was 24.5% ± 9.0% lower ($p < 0.05$) in COT arteries of OB than C ewes. Content of total insulin receptor, total IGF-1 receptor and their phosphorylated forms were similar across dietary groups. **Conclusions:** These data demonstrate that the phosphorylation of AMPK and its down-stream target ACC were reduced in COT arterial tissue of OB mothers. This reduction in AMPK activity was associated with decreased phosphorylation of IRS-1 at Ser789, which is expected to reduce the PI3K/Akt and MAPK/ERK1/2 activation mediated by IRS-1, thus decreasing down-stream insulin/IGF-1 signaling in OB ewes despite elevated glucose, insulin and IGF-1 concentrations in fetal blood.

ENHANCED ADIPOGENESIS AND DECREASED AMPK ACTIVITY IN FETAL MUSCLE OF OBESE, OVERNOURISHED PREGNANT SHEEP

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The increasing prevalence of overweight and obese women of childbearing age is a growing public health concern. Persistent health effects on offspring of obese women, including pre-disposition to obesity and diabetes have been observed, but remain poorly defined. Adipogenesis in fetal muscle is initiated around mid-gestation.

Objectives: Cultured 3T3 cells were used to test the role of AMP-activated protein kinase (AMPK), a key mediator of lipid metabolism, in adipogenesis by employing 5-aminoimidazole-4-carboxamide 1-beta-d-ribonucleoside (AICAR), a specific activator of AMPK. In addition, alteration of adipogenesis was assessed in fetal muscle in response to maternal obesity and over-nutrition, and possible mechanisms were explored. **Methods:** 3T3 cells were cultured in adipogenic medium with or without AICAR. For the in-vivo study, non pregnant ewes were randomly assigned to a control (C,100% of NRC recommendations, n = 7) or obesogenic (OB, 150% of NRC, n = 7) diet from 60 days before to 75 days after conception when ewes were euthanized. The fetal *longissimus dorsi* (Ld) muscle was collected and weighed, and key signaling proteins were measured in frozen tissue by western analysis. Cryosections were evaluated for muscle fiber density or stained for intramyocellular fat by Oil-Red O. **Results:** Activation of AMPK by AICAR, dramatically reduced adipogenesis in 3T3 cells incubated in an adipogenic medium, with 0.1 mM AICAR treatment decreasing adipogenesis by 69.95 ± 2.4% and 1 mM AICAR by 91.79 ± 1.9%. This reduction in adipogenesis in 3T3 cells was correlated with the activation of AMPK and Acetyl-CoA carboxylase (ACC), a down-stream target of AMPK and a key enzyme limiting lipogenesis. The phosphorylation of both AMPK and ACC increased by more than 2 fold in response to 1 mM AICAR treatment. The weights of OB fetuses were approximately 30% heavier ($P < 0.05$) than fetuses from C ewes. The weight of the Ld muscle was greater ($P < 0.01$) for OB than for C fetuses (2.18 ± 0.15 versus 1.60 ± 0.07 g, respectively). As shown by Oil-Red O staining, the content of intramuscular fat was 2 fold greater ($P < 0.05$) in OB Ld muscle than C Ld muscle. The density of muscle fibers was 14.2 ± 0.5% lower ($P < 0.05$) in OB muscle compared to C muscle. The expression of PPARγ, a marker of adipogenesis, was 18.2 ± 3.4% higher ($p < 0.05$) in the fetal muscle from OB compared to C pregnancies, but phosphorylated AMPK was down-regulated ($P < 0.05$) by 14.9 ± 4.2% ($p < 0.05$) in OB versus C fetal muscle. **Conclusions:** These data show that OB pregnancy altered fetal muscle development by promoting adipogenesis in fetal muscle. This enhanced adipogenesis is correlated with the down-regulation of AMPK activity in OB fetal muscle, showing that AMPK might have an important role in the regulation of fetal muscle development.

LOW-SODIUM DIET DURING RAT GESESTATION: IS FETAL GROWTH LINKED TO PLACENTAL HYPOXIA?

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Objectives: We have developed an animal model of intrauterine growth restriction (IUGR) by giving a low-sodium diet to dams over the last week of gestation. We observed in the mother a reduction of circulating volume expansion, diminished increase in uterine arcuate artery diameter and lower placental weights, suggesting an altered uteroplacental perfusion. We thus propose that IUGR observed in our model might be associated with placental hypoxia. **Methods:** During the last week of gestation, half of the dams received a low-sodium diet. On day 22 (term = 23 days), rats were sacrificed and placentas were collected and snap frozen. The vascular growth promoter (VEGF) can be induced by hypoxia and stimulates nitric oxide synthases (endothelial, eNOS and inductible, iNOS) and glucose transporters (GLUTs). Gene expression of VEGF and its receptors as well as GLUTs was thus evaluated by semi-quantitative PCR whereas the protein expression of VEGF, NOSs and GLUTs was measured by ELISA or Western blot. VEGF localisation was also performed by immunohistochemistry. Measurement of oxygen metabolites such as superoxide anion (O_2^-) can be used as sensor of oxygen content. Systems are protected from this anion by the activity of superoxide dismutase (SOD) and catalase which were evaluated by commercial colorimetric assays. In order to check a possible interaction between nitric oxide and O_2^-, the presence of nitrotyrosine proteins was detected by slot blot with a specific antibody. Since chronic hypoxia can induce apoptosis, protein expression of apoptosis-related protein (pro-apoptosis proteins: Bax and p53; anti-apoptosis proteins: Bcl-2 and Bcl-X_L) was measured by Western blot. As p53 expression is regulated by methylation, its promoter methylation was evaluated by PCR. **Results:** We observed in placentas from salt-restricted dams 1) decreased VEGF gene expression but increase in its protein levels which is supported by immunohistochemical analysis, 2) higher levels of VEGFR1 mRNA, 3) augmentation in eNOS protein without changes in iNOS expression, 4) reduction in GLUTs levels that is only statistical for GLUT1, 5) no modification in SOD and catalase activity as well as nitrotyrosine protein abundance, 6) increased p53 protein expression possibly due to the slight decrease in its promoter methylation and 7) increased apoptotic index as reflected by the higher ratio of Bax/Bcl-2 as well as Bax-Bcl-X_L protein levels. **Conclusion:** Our data suggest that reduced placental and fetal growth observed in our model might be mediated, in part, through moderate level of placental hypoxia and apoptosis. However, other works performed in our laboratory indicate that IUGR fetuses are not hypoxic, indicating that placental hypoxia is not sufficient to compromise their oxygen support.

INFLUENCE OF MATERNAL NUTRITION ON mRNA EXPRESSION OF ANGIOGENIC FACTORS AND RECEPTORS IN SKELETAL MUSCLE OF ADOLESCENT SHEEP

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Objectives: Objectives were to examine effects of maternal nutrient restriction and dietary selenium (Se) on mRNA expression of angiogenic factors and their receptors in fetal skeletal muscle. **Methods:** Targhee-cross ewe lambs (n = 36) were randomly assigned to plane of nutrition treatments (control [CON, 100% of requirements] or restricted [RES, 60% of controls]) and dietary Se treatments (adequate Se [ASe, 6 μg/kg BW] or high Se [HSe, 80 μg/kg BW]) from Se-enriched yeast. Selenium treatments were initiated 21 d prior to breeding and restriction treatments on d 64 of gestation. Diets contained 16% crude protein and 2.12 Mcal/kg metabolizable energy (DM basis). Nutrient requirements were based on ewe BW, and nutrient intake was adjusted every 2 weeks to account for changes in BW. Tissues were harvested on d 135 ± 5 of gestation. Fetal Longissimus muscle (~5 g) was sampled and DNA, RNA, and protein concentrations were determined. Quantitative real-time reverse transcription-polymerase chain reaction and ovine-specific probe and primer sets were used to determine mRNA expression of major angiogenic factors and their receptors relative to the sample's internal 18S RNA. **Results:** As previously reported, muscle from lambs whose dams were fed RES-HSe diet had greater DNA ($P = 0.043$) and RNA ($P < 0.10$) than CON-ASe, CON-HSe, and RES-ASe. Total protein concentration was lower in RES-ASe ($P = 0.017$) than either CON-ASe or CON-HSe. Consequently, protein:DNA was smaller in RES treatment group than CON ($P = 0.009$). Nutrient restriction upregulated mRNA expression of angiopoietin-1 (*ANG1*; $P = 0.08$). High Se treatments upregulated mRNA expression of the receptor neuropilin-1 ($P = 0.06$) and downregulated the expression of basic fibroblast growth factor ($P = 0.06$). Nutrient restriction or selenium supplementation did not alter mRNA expression in fetal muscle of vascular endothelial growth factor (*VEGF*) or hypoxia inducible factor-1α transcription factor. Additionally, treatment groups did not alter the mRNA expression for receptors *FGFR2, FLT1,* or *KDR* ($P > 0.10$). **Conclusions:** Maternal dietary treatments induced changes in expression of some angiogenic factors and receptors in fetal skeletal muscle, which may be a mechanism that impacts muscle vascularization, thus impacting skeletal muscle growth. The relationship between angiogenic factor and receptor expression, fetal muscle vascularity, and muscle growth are currently being investigated.

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THYROID HORMONE IS A POTENT REGULATOR OF FETAL SHEEP CARDIOMYOCYTE PROLIFERATION

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Objectives: Maturation of cardiomyocytes in the fetus proceeds from a "proliferating" to "terminally differentiated" non-proliferating state as gestation proceeds. Thyroid hormone (T_3) is a key regulator in organ development and thought to play a major role in tissue maturation. Its concentration increases from late-gestation to birth. In a previous study of cardiomyocytes from late-gestation sheep fetuses (135 days gestational age (dGA) where term is ~150 dGA) we found a significant decrease in proliferation and changes in cell cycle markers due to T_3 administration (Chattergoon *et al. J Endocrin* 192, 2007). From these data, we hypothesized that T_3 drives maturation of fetal cardiomyocytes by decreasing their proliferative capacity *in vitro*. The current study investigates cardiomyocytes from younger animals (100 dGA) to better understand T_3 in the heart from a developmental standpoint. **Methods:** Ewes were euthanized by intravenous injection of a commercial solution of sodium pentobarbital (Euthasol, ~65 mg/kg, Virbac, TX). Fetal hearts were excised, weighed and enzymatically dissociated by retrograde perfusion of a collagenase and protease solution. The cardiomyocytes were isolated from left and right ventricle and incubated in culture with a range of physiological to pharmacologic T_3 concentrations (0.37, 0.75, 1.5, 3, 10, 100 nM) and BrdU (10 μM) for 48 hours to study proliferation under different serum conditions. **Results:** Basal BrdU uptake in response to serum free (SF) media increases to 8–10% in 100 dGA cardiomyocytes compared to 1–2% in 135 dGA cardiomyocytes. BrdU uptake in response to 10% serum media increases to 15–20% from 8–10% in 135 dGA cardiomyocytes. T_3 significantly decreases BrdU uptake in serum media 2-fold. T_3 in SF media did not increase or decrease BrdU uptake. Considering the powerful suppressive effect T_3 has in the presence to serum media in the younger cells it offers insights to T_3 signaling at an age when T_3 concentrations are low *in vivo*. **Conclusions:** 1) T_3 inhibits fetal cardiomyocyte proliferation *in vitro* as measured by BrdU uptake, and 2) T_3 exhibits differential effects depending on serum concentration in cardiomyocytes isolated from younger animals. These data suggest T_3 is a potent regulator of cardiomyocyte proliferation and maturation. High concentrations of thyroid hormone early in development may lead to a "mature" cardio-phenotype at an inappropriate point in gestation, resulting in fewer cardiomyocytes at birth.

SERUM LEVELS OF IGF-I AND IGFBP-3 IN SMALL AND APPROPRIATE FOR GESTATIONAL AGE NEWBORN INFANTS

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Objectives: To evaluate the influence of IGF-I and IGFBP-3 in growth of small for gestational age newborn infants. **Methods:** Small for gestational age and appropriate for gestational age newborn infants were paired by sex and gestational age, and followed from birth to the age of term. Exclusion criteria: congenital malformation,congenital infections and genetic syndromes. Growth was evaluated by weight for age z score. Blood was collected in the first day of life and at the age of term. The SAS statistic software, version 9.01, was used for the analyses. The values of IGF-I and IGFBP-3 were log-transformed. Informed consent was obtained from the families and the study was approved by the Ethics Committee. **Results:** Fifty-four newborn infants were studied, 28 small for gestational age (17 preterm) and 26 appropriate for gestational age (15 preterm). There were no significant differences between the two groups at baseline, except for birth weight, birth length, head circumference and weight for age z score at birth ($p < 0.0001$). Also there was no differences in neonatal course between the groups ($p > 0.05$). At birth IGFBP-3 levels were lower in small for gestational age (SGA) compared with appropriate for gestational age (AGA) babies ($541.95 \pm 295.89 \times 682.43 \pm 287.09$, $p = 0.008$) but IGF-I levels were similar in both groups ($2.78 \pm 2.37 \times 3.99 \pm 3.04$, $p = 0.39$). At age of term IGF-I and IGFBP-3 were significantly lower in SGA babies (IGF-I: $13.53 \pm 12.34 \times 50.09 \pm 24.04$, $p = 0.005$; IGFBP-3: $678.59 \pm 185.01 \times 1323.9 \pm 361.96$, $p < 0.0001$). The increased level of IGF-I and IGFBP-3 from birth to term, in preterm babies, was significantly greater in the AGA group. The IGF-I difference (term-birth) found was 9.97 ± 11.48 in SGA group and 42.64 ± 41.78 in AGA group ($p < 0.0001$). For IGFBP-3 these differences were 203.74 ± 171.45 and 668.43 ± 446.18, respectively ($p < 0.0001$). IGF-I at birth was significantly associated with weight z score at term ($p = 0.03$), adjusted by SGA and days of life, but not IGFBP-3 ($p = 0.24$). In preterm babies, neither the variation of IGF-I nor IGFBP-3 from birth to term associated with weight for age z score at term age ($p = 0.11$ and 0.90, respectively), although there was a greater reduction in the number of babies studied ($n = 24$ and 25, respectively). **Conclusions:** This study have not found IGF-I deficiency in SGA babies at birth. The greater increase in IGF-I and IGFBP-3 levels from birth to term in both preterm SGA and AGA babies demonstrated a period of important growth. In spite of this, SGA preterm babies kept lower levels of both factors. In addition IGF-I level at birth was associated with lower weight z score at term, pointing to an endocrine disturb, which could reflect in long time growth.

MYOCYTE PROLIFERATION CONTRIBUTES TO ANEMIA-INDUCED FETAL CARDIAC ENLARGEMENT

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Objectives: Chronic anemia increases the workload of the growing fetal heart, leading to cardiac enlargement. In order to determine by what cellular process(es) cardiac mass is increased, we measured cardiomyocyte sizes, terminal differentiation, and tissue volume fractions in hearts from control and anemic fetal sheep. **Methods:** Experiments were conducted in chronically catheterized fetal sheep for ~9 days to obtain intravascular pressures and arterial blood samples. Eleven fetuses were phlebotomized to cause severe anemia during the experiment, and were compared to 12 control fetuses. At postmortem, hearts were either dissociated or fixed for morphometric analysis. **Results:** Daily isovolumetric hemorrhage reduced fetal hematocrit from a baseline value of 35% to 16% on the final day ($p < 0.001$ different from baseline and age-matched controls). Anemic did not have a statistically significant effect on mean arterial pressure, central venous pressure or heart rate. Heart weight was increased by 38% in anemic fetuses compared to controls ($p < 0.0001$), although the groups had similar body weights. The ratio of cardiac dry- to wet-weight was not different between anemic and control fetuses. Cardiomyocytes from anemic fetuses were not larger than those of control fetuses. There were no statistically significant differences between groups of fetuses in the volume percent of the various tissue fractions or the degree of terminal differentiation. **Conclusions:** By ruling out other modes of growth we conclude that cardiomyocyte proliferation contributed to anemia-induced cardiac enlargement. Fetal anemia might result in an overabundance of cardiomyocytes at birth, the consequences of which are unknown.

THE INTRAUTERINE GROWTH RESTRICTION-INDUCED DELAY IN CARDIOMYOYCTE BINUCLEATION IS RELATED TO HYPOXIA NOT HYPOGLYCEMIA

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Objectives: Recent studies show that intrauterine growth restriction (IUGR) causes a delay in terminal differentiation of cardiomyocytes in the sheep fetus. The methods of inducing intrauterine growth restriction cause chronic hypoxia and hypoglycaemia but the underlying cause of the altered cardiomyocyte development remains unclear. **Methods:** Placental and hence fetal growth restriction was induced in fetal sheep by removing the majority of caruncles in the ewe before mating (placental restriction, PR). Vascular surgery was performed on 17 Control and 11 PR fetuses at 110–125 d gestation (term = 150 ± 3 d). PR fetuses with a mean gestational $PO_2 < 17$ mmHg were defined as hypoxic. At *post mortem* (<135 or >135 d), fetal hearts were collected, and cardiomyocytes isolated and fixed. Cardiomyocytes were stained with methylene blue to visualise the nuclei and the proportion of mononucleated cells was counted. **Results:** PR resulted in chronic fetal hypoxia, IUGR, elevated plasma cortisol concentrations and reduced glucose concentrations. Although there was no difference in relative heart weights between Control and PR fetuses, there was an increase in the proportion of mononucleated cardiomyocytes in PR fetuses. There was a significant relationship between mean gestational PO_2 and the percentage of mononucleated cardiomyocytes in both the right and left ventricle. There was no relationship between plasma glucose and the percentage of mononucleated cardiomyocytes. **Conclusions:** The increase in the relative proportion of mononucleated cardiomyocytes in the growth restricted fetus is likely due to chronic hypoxia rather than hypoglycaemia.

A MOUSE MODEL IN WHICH HIGH FAT DIET PRIOR TO AND DURING GESTATION RESULTS IN FETAL OVERGROWTH

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Objective: More than 50% of all pregnant women in the US today are overweight or obese, representing one of the most challenging health problems in obstetrics. The baby of the obese woman is often large at birth, which is associated with traumatic birth injuries and an increased risk to develop obesity, diabetes and hypertension in childhood and later in life. The mechanisms underlying fetal overgrowth in pregnant women with obesity are, however, largely unknown, and the lack of relevant animal models has severely hampered progress in this area. The aim of this study was to establish a mouse model of obesity/high fat diet in pregnancy that display similarities with the human condition including (1) a metabolic profile similar to that of obese pregnant women, i.e. high levels of insulin, leptin, IGF-I and cytokines, and decreased levels of adiponectin, and (2) fetal overgrowth. **Method:** C57BL/6J female mice were fed control (11% of energy from fat) or high fat (32% of energy from fat) diets ad libitum for 8 weeks prior to mating and during gestation. At gestational day 18.5 maternal blood samples were obtained and fetuses and placentas were collected and weighed. Mean placental and fetal weights were calculated for the individual litters and these values were used in further analysis. **Results:** No significant differences were observed in maternal pre-pregnancy bodyweight, total caloric intake, weight of the dam at E18.5 or litter size between treatment groups. However, fetuses from dams on the high fat diet were 30% larger ($1.06 +/- 0.15$ g, $n = 6$ litters) than fetuses from dams on the control diet ($0.73 +/- 0.08$ g, $n = 5$ litters, $p < 0.05$). In contrast, placental weights were not significantly different between treatment groups resulting in higher fetal-placental ratios in the high fat fed group ($p < 0.05$). **Conclusion:** This study establishes, for the first time, a mouse model in which high fat diet prior to and during gestation results in fetal overgrowth. The higher fetal-placental weight ratio in the high fat diet animals is compatible with an up-regulation of placental nutrient transport.

REDUCED SYSTOLIC PRESSURE LOAD, *IN VIVO*, DECREASES CELL CYCLE ACTIVITY IN THE FETAL SHEEP HEART

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Objectives: Increased systolic pressure load accelerates fetal heart growth by both hypertrophy and hyperplasia. We have previously shown that elevated systolic pressure load increases cardiomyocyte size and cell cycle activity of near-term fetal sheep (Jonker *et al.* Am J Phyiol 292, 2007). The effects of reduced systolic pressure load on fetal cardiac growth are not known. Based on the current understanding of load modulated fetal cardiac growth we hypothesized that reduced systolic pressure load would decrease both hypertrophic and hyperplastic fetal cardiac growth. **Methods:** Five fetal sheep were instrumented to measure arterial pressure (AP), central venous pressure (CVP) and heart rate (HR). After baseline hemodynamic measurements enalaprilat was infused (345ug/day) to lower fetal arterial pressure and thus the systolic load of the heart. After 8 days (134 days gestation) the fetal hearts were harvested, weighed and enzymatically dissociated. Dissociated cardiomyocytes were analyzed for cell size, maturational state (proportion of binucleated myocytes) and cell cycle activity (as measured by positive staining of cells for Ki-67). The results for the reduced systolic load group (E) were compared to results obtained from normal arterial pressure group (C) of the same gestational age. **Results:** Over the 8 day treatment period, AP decreased from 42 ± 2 mm Hg to 23 ± 3 mm Hg; CVP remained the same (2.3 ± 0.5 vs 2.7 ± 1.2 mm Hg); HR decreased from 173 ± 9 to 136 ± 11 bpm. The heart-to-body weight ratio was 5.5 ± 0.6 g/kg in the experimental and 6.0 ± 0.3 g/kg in the control group (heart weights: 20.9 ± 2.9 (E) vs 21.3 ± 2.8 g (C); body weights: (E) 3.8 ± 0.7 vs 3.5 ± 0.3 kg (C)). Left ventricle (LV) myocyte measurements were as follows: mononucleate myocyte length: 63.2 ± 4.5 (E) vs 61.6 ± 2.3 um (C); mononucleate myocyte width: 8.7 ± 0.4 (E) vs 9.3 ± 0.3 um (C); binucleate myocyte length: 83.3 ± 4.9 (E) vs 79.9 ± 1.7 um (C); binucleate myocyte width: 9.7 ± 0.5 (E) vs 10.6 ± 0.4 um (C). Similar to these LV data, right ventricle (RV) myocyte size was not different between treatment groups. The portion of binucleated myocytes in the LV or RV was not different in the experimental group compared to the control group. The percentage of Ki-67 positive mononucleated myocytes was decreased in both the LV ($1.5 \pm 1.1\%$ (E) vs $12.0 \pm 4.2\%$ (C)) and RV ($1.2 \pm 0.8\%$ (E) vs $10.4 \pm 2.5\%$ (C)) of experimental fetuses. **Conclusions:** Reduced cardiac systolic load had little effect on cardiac myocyte size while decreasing cell cycle activity, suggesting a major effect on hyperplastic growth and no effect on hypertrophic growth. As cardiomyocyte proliferation is nearly complete at birth in mammals, a significant decrease in hyperplastic growth *in utero* could reduce the number of cardiomyocytes present in the heart at birth.

SYNERGISTIC INDUCTION OF 11β-HYDROXYSTEROID DEHYDROGENASE TYPE 1 EXPRESSION BY CORTISOL AND INTERLEUKIN-1B IN HUMAN FETAL LUNG FIBROBLASTS

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Objectives: Glucocorticoids act through binding to its cytosolic glucocorticoid receptor (GR), serving as crucial hormones in fetal lung maturation. The amount of cortisol available to its receptors is increased by the pre-receptor enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β- HSD1) which converts biologically inactive cortisone to active cortisol. Glucocorticoids and pro-inflammatory cytokines are known to induce 11β-HSD1 expression in a number of tissues, but the action of glucocorticoids and proinflammatory cytokines in the regulation of 11β-HSD1 expression has not been addressed in human fetal lung fibroblasts where 11β-HSD1 is reported to to be exclusively localized in the lung. Therefore, we examined the actions of cortisol and interleukin-1β (IL-1β) and their interaction on 11β-HSD1 expression in human fetal lung fibroblasts (HFL-1) and characterized the underlying mechanisms. **Methods:** 11β-HSD1 mRNA level in cultured human fetal lung fibroblasts treated with cortisol and IL-1β was measured with real time PCR. The roles of GR and C/EBPs in the effect of cortisol and IL-1β were studied using GR antagonist and transfection of plasmid carrying C/EBP-specific dominant-negative gene (CMV500-A/CEBP) respectively. **Results:** Both cortisol ($10^{-8} \sim 10^{-6}$ M) and IL-1 β (0.1 ~ 10 ng/ml) induced 11β-HSD1 mRNA expression in a dose-dependent manner, which could be blocked by mRNA transcription inhibitor 5,6-dichlorobenzimidazole riboside (75 μM), suggesting the induction is dependent on ongoing transcription. The induction of 11β-HSD1 mRNA expression by cortisol (10^{-6} M) was synergistically increased by co-treatment with IL-1β and this synergistic effect was increased with increasing doses of IL-1β (0.1 ~ 10 ng/ml). On the contrary, the induction of prostaglandin H synthase-2 (PGHS-2) expression by IL-1β was concurrently inhibited by cortisol, suggesting a different interacting mechanism was utilized by cortisol and IL-1β in the synergistic induction of 11β-HSD1 expression. Glucocorticoid receptor antagonist RU486 (10^{-6} M) was able to block the induction of 11β-HSD1 by cortisol. Transfection of the cells with C/EBP-specific dominant-negative expression (AC/EBP) plasmid could attenuate the induction of 11β-HSD1 mRNA expression by either cortisol or IL-1β. These observations suggest that the induction of 11β-HSD1 expression by cortisol is a GR dependent process involving C/EBPs, which also mediates the induction of 11β-HSD1 expression by IL-1β. **Conclusions:** We have shown in this study that cortisol and IL-1β could synergistically induce the expression of 11β-HSD1 in human fetal lung fibroblasts. With more cortisol produced, these effects might provide either a self-resolving mechanism for inflammation or a mechanism for enhancing fetal lung maturation when the fetus under the threat of infection-induced preterm labor.

INFLUENCE OF MATERNAL NUTRITION ON MRNA EXPRESSION OF ANGIOGENIC FACTORS AND RECEPTORS IN MAMMARY GLAND OF ADOLESCENT SHEEP

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Objectives: Objectives were to examine the effects of nutrient restriction and dietary Se on mRNA expression of major angiogenic factors and their receptors in mammary tissue of primigravid sheep. **Methods:** Targhee-cross ewe lambs (n = 36) were allotted randomly to one of four treatments in a 2 × 2 factorial design. Treatments were plane of nutrition (control [CON; 100% of requirements] vs. restricted [RES; 60% of controls]) and dietary Se (adequate Se [ASe; 6 μg/kg BW] vs. high Se [HSe; 80 μg/kg BW]) provided as Se-enriched yeast. Selenium treatments were initiated 21 d prior to breeding and plane of nutrition treatments were implemented on d 64 of gestation. Nutrient requirements were based on ewe body weight, and nutrient intake was adjusted every 2 weeks to account for changes in body weight. Tissues were harvested on d 135 ± 5 of gestation. Mammary tissue was sampled (~5 g) and analyzed for concentration of RNA, DNA, and protein. Quantitative real-time RT-PCR and ovine-specific probe and primer sets were used to determine mRNA expression of major angiogenic factors and their receptors relative to the sample's internal 18S RNA. **Results:** Mammary gland mass was decreased in RES ewes relative to that of CON ($P = 0.01$), although no differences were apparent when mass was expressed as a proportion of empty body weight ($P = 0.15$). Total protein content of the mammary gland was lower in RES ewes ($P = 0.01$). The ratio of protein:DNA and concentrations of RNA, DNA, and protein per gram of tissue were not altered by dietary treatment ($P \geq 0.16$). Nutrient restriction upregulated the mRNA expression of vascular endothelial growth factor (*VEGF;* $P < 0.01$) and nitric oxide synthase 3 (*eNOS;* $P = 0.06$). Both nutrient restriction ($P = 0.05$) and high Se ($P = 0.10$) decreased expression of angiopoietin 1 (*ANGPT1*). Additionally, mid to late gestation nutrient restriction enhanced expression of endothelial tyrosine kinase (*TIE2,* an ANGPT1 receptor; $P = 0.07$) and neuropilin 1 (*NRP1;* $P = 0.09$). Expression of two major VEGF receptors, kinase insert domain receptor (*KDR*) and vascular endothelial growth factor/vascular permeability factor receptor (*FLT1*), were not affected ($P \geq 0.25$) by nutrient restriction or Se supplementation. **Conclusion:** Nutrient restriction induced changes in angiogenic factor and receptor expression in the mammary gland of late-term primigravid sheep, which may be a mechanism by which nutrient delivery to offspring is enhanced during conditions of food scarcity. The relationship among angiogenic factor and receptor expression, mammary gland vascularity, and colostrum yield and quality is currently being investigated in our laboratory.

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MARGINAL COPPER DEFICIENCY IN PREGNANCY: VASCULAR RESPONSES IN DAMS AND PROGENY

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Objectives: Copper (Cu) is essential in defense against oxidative stress and to development of connective tissue in the heart and blood vessels. The long- term effects of marginal Cu deficiency on vascular function during pregnancy have not been characterized previously. In this study, the vascular consequences of marginal Cu deficiency were determined by measurement of contractile and relaxation responses in mesenteric arteries of dams and their offspring. Residual effects perpetuated to a subsequent generation of offspring were determined in progeny of dams and sires with intrauterine exposure to marginal Cu deficiency during development and/or lactation. **Methods:** Pregnant dams were fed an AIN93G diet beginning 3 weeks before conception and remained on the diet throughout lactation until postnatal day (PND) 21. Dams consuming diets containing 1 mg Cu/kg were marginally Cu deficient (CuD; n = 7); dams fed a diet containing 6 mg Cu/kg served as controls (CuA; n = 8). To define the critical developmental window, fifty percent of pups born to CuD dams were cross-fostered to CuA dams and vice versa on PND 1. Pups that were born to and remained with their CuD birth mothers until weaning were considered CuD first generation (F1) offspring; pups born to and remaining with Cu adequate birth mothers served as CuA F1 controls. After weaning, all offspring were transitioned to rat chow containing adequate amounts of Cu. At reproductive maturity, F1 offspring were mated within groups to determine perpetuation of vascular effects resulting from Cu deficiency in a second generation (F2). A small wire myograph was used to determine mesenteric arterial responses to vasoconstrictors (phenylephrine [PE], potassium chloride [KCl]) and endothelium-dependent and -independent relaxants (acetylcholine [ACh]) and sodium nitroprusside [SNP], respectively) in dams on PND 21 and in offspring groups at 9 weeks of age (n = 5–7/group). Group differences were determined by students *t* test or ANOVA with Tukey post hoc with a level of <0.05 considered significant. **Results:** There were no significant differences in vasoconstrictor or vasorelaxant responses in dams. Among F1 male offspring, vasoconstrictor responsiveness to KCl was increased when Cu deficiency was limited to the lactation period ($p < 0.05$). Relaxation responses were impaired among female F1 offspring with Cu deficiency limited to lactation, as compared to deficiency during both intrauterine development and lactation (endothelium-dependent and independent, $p < 0.05$), to offspring with intrauterine exposure to Cu deficiency coupled with adequate Cu during lactation (endothelium-dependent, $p < 0.05$); and to control (endothelium-independent, $p < 0.05$). Among F2 offspring, there were no differences in vasoconstrictor or vasorelaxant responsiveness between groups. **Conclusions:** Cu deficiency during early postnatal nutrition leads to altered mesenteric artery responsiveness in a gender-specific manner. Alterations in vascular function were not perpetuated to a second generation of offspring.

MATERNAL PROTEIN RESTRICTION IN SWINE: INCREASED AORTIC NOX 4 MRNA IN BOTH FETAL AND JUVENILE OFFSPRING PARALLELS INCREASED MESENTERIC NADPH OXIDASE-DEPENDENT ANGII REACTIVITY IN JUVENILES

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In microswine, isocaloric Maternal Protein Restriction (MPR: 1% vs 14%) in late gestation/early lactation yields asymmetric growth restriction, hyperphagia, and accelerated growth over 6–12 wks, but not obesity at Wk 12, in Low-Protein Offspring (LPO) vs Normal Protein Offspring (NPO). At age 3–5 mo (juveniles), LPO exhibit restraint-stress-induced (but not basal) hypertension in vivo and mesenteric small-artery hyperreactivity to KCl, NorEpi, and AngII ex vivo, without differences in vascular wall geometry. AngII vascular reactivity was normal in near-term fetal LPO but increased in juvenile LPO, the latter mediated by exaggerated AT1R contractile signaling through an NADPH oxidase/EGF receptor-dependent pathway. Nox4 is a major catalytic subunit of NADPH oxidase in vascular smooth muscle.

Objectives: We sought to learn whether increased expression of Nox 4 explained AngII hyperreactivity in juvenile vasculature and whether similar upregulation of aortic Nox 4 in fetal LPO supported NADPH oxidase as a primary target of nutritional programming. **Methods:** Abdominal aortas were harvested from anesthetized near-term fetal LPO and NPO on GD 113 of 115 via c-section and from terminally anesthetized juvenile offspring. Tissue was snap-frozen (fetal) or incubated overnight in RNA later (juvenile), and stored at −80. After RNA extraction (QIAgen RNeasy) and reverse transcription, cDNA was amplified using pig-specific primers for Nox4 and GAPDH with SYBRGreen® in an Applied Biosystems PCR system. Concurrent standard curves for each mRNA were generated using a pooled sample; results for each pig were referenced to the relevant standard curve, then expressed as ratio of Nox4:GAPDH cDNA. **Results:** In near-term fetal offspring, aortic Nox4 mRNA expression in LPO (1.94 ± 0.77, n = 6) was increased 4-fold over fetal NPO (0.46 ± 0.25, n = 6, $p < .01$). Similarly, in juvenile offspring, Nox 4 mRNA in LPO (1.21 ± 0.38, n = 9) remained elevated vs juvenile NPO (0.71 ± 0.30, n = 11, $p < .01$). **Conclusion:** We conclude that, given fetal upregulation of Nox4 mRNA without functional hyperactivity, together with persistent juvenile elevation of Nox4 coupled with vascular hyperreactivity to AngII, NADPH oxidase may be a primary target of nutritional programming by MPR. We propose that low oxygen availability during MPR in utero leads to compensatory activation of oxygen dependent signaling pathways to sustain critical morphogenic growth; Nox4/NADPH oxidase upregulation persists postnatally and, with restored oxygen availability at birth, generates excessive reactive oxygen species, thereby mediating enhanced functional responses to pressors which utilize this key downstream signaling relay station.

POPULATION SUSCEPTIBILITY TO SGA AND PREECLAMPSIA REDUCE UTERINE ARTERY BLOOD FLOW VIA DIFFERENT MECHANISMS

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Objective: Compared to healthy Europeans (EUR) residing at high altitude, babies born to healthy native Andeans (AND) are relatively protected from hypoxia-associated reductions in birth weight. We asked whether preeclampsia (PE) in native Andeans and EUR ancestry reduce birth weight by different physiologic mechanisms. **Methods:** We studied 155 normal AND, 32 Andeans with PE (AndPE), and 38 EUR between 20–36 wk gestation, and calculated blood flow as the product of mean velocity times vessel cross-sectional area, measured using Doppler ultrasound. Babies weighing below the 10th percentile adjusted for age and sex were classified as small for gestational age (SGA), and pre-term if <37 wk. **Results:** Uterine artery (UA) blood flow in EUR was one-third that seen in normal AND ($p < 0.0001$), due to smaller vessel diameters and slower mean velocities. EUR women also had ~20% lower common (CI) and external iliac (EI) artery blood flow ($P < 0.01$), due also to smaller vessels and slower velocities. However, the ratios of UA/CI and UA/EI blood flow were 40% lower than in normal AND, indicating a disproportionate reduction in UA blood flow. In AndPE, UA blood flow was also only one-third that seen in normal AND women at 24–28 weeks gestation ($P < 0.001$), due entirely to slower mean velocity, with differences diminishing near term. CI and EI blood flow were ~40% higher ($P < 0.01$) in AndPE compared to normal AND, but the ratios of UA/CI and UA/EI flow were ~45% lower, indicating a marked reduction in the fraction of blood flow distributed to the UA. Differences in right and left UA flow were observed in Andean (but not EUR) women. Left UA flow was 25% higher than the right in normal AND, but was markedly reduced in AndPE, so that right UA flow was 15% greater than the left. Both PE and EUR ancestry increased the frequency of SGA (AndPE = 52%, EUR = 23%, vs. AND = 15%, both $P < 0.001$) and pre-term births (AndPE = 28%, EUR = 16%, vs. AND = 5%, both $P < 0.001$). Similar head circumference in all groups and greater middle cerebral to umbilical artery peak flow in AndPE and EUR fetuses compared to normal AND ($p < 0.001$) were consistent with asymmetric fetal growth restriction in the AndPE and EUR babies. **Conclusions:** EUR population ancestry and PE in native Andeans both reduce UA blood flow and fetal growth at high altitude, but fetal brain blood flow is preserved. Greater UA, CI, and EI diameters during pregnancy appear to be fixed, perhaps genetic characteristics of Andeans vs. EUR women, indicating greater uteroplacental vascular remodeling and/or dilation during pregnancy that enable increased UA blood flow. Lower UA flow velocities in AndPE are consistent with increased vascular resistance due to impaired trophoblast remodeling, suggesting that the mechanisms by which PE reduces UA flow operate downstream of those of population ancestry. (NIH-HL079647-01S1).

ALTITUDE DECREASES AND ANDEAN ANCESTRY INCREASES TNF ALPHA LEVELS DURING PREGNANCY

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Objective: Tumor necrosis factor alpha (TNFα) is involved in inflammatory as well as other pregnancy-related processes. Whereas TNFα levels decline during normal pregnancy, likely as part of a generalized immunosuppression, 3rd trimester levels are higher in the pregnancy complications of preeclampsia (PE) and intrauterine growth restriction (IUGR). Residence at high altitude raises the frequency of both complications, likely due to chronic hypoxia influencing maternal vascular responses to pregnancy. The role of TNFα in these altitude effects is unknown. Because women of multigenerational (Andean) vs. shorter duration (European) high-altitude residence have greater uterine artery blood flow and their babies protected from IUGR, we hypothesized that Andean compared with European women at high altitude had a greater pregnancy-associated decline in TNFα levels. **Methods:** We studied 125 normal women, comprising 29 Andean and 39 European low-altitude (400 m) and 27 Andean and 30 European high-altitude (3600 m) residents. TNFα levels were determined by ELISA in plasma obtained from peripheral venous blood at weeks 20 and 36 of gestation, and 4 mo postpartum as index of the nonpregnant state. Data were analyzed using 1 and 2- way ANOVA. **Results:** In the European women, pregnancy at low altitude tended to reduce TNFα levels ($p = 0.06$) and values declined at high altitude at both weeks 20 and 36 (1.2 ± 0.3 pg/mL and 1.6 ± 0.3 respectively) compared with postpartum (2.7 ± 0.3 pp, both $p < 0.01$). In contrast, there was no effect of pregnancy on TNFα levels in the Andean women at either low or high altitude. Residence at high altitude increased and Andean ancestry decreased TNFα levels in the nonpregnant as well as the pregnant state (2-way ANOVA, both $p < 0.05$). **Conclusion:** Both altitude and ancestry affected TNFα levels during pregnancy but in ways that differed from those hypothesized. Rather than pregnancy producing a greater fall in TNFα levels, the pregnancy-associated decline was reduced and the Andean women had higher TNFα levels than the European subjects under all measurement conditions. Also unexpected was that high-altitude residence raised TNFα levels independent of ancestry, an effect which was opposite of that seen in a previous Colorado report (Coussons-Read ME et al, Am J Reprod Immunol 48:344–354, 2002). Such results may imply that Andean ancestry and/or high-altitude residence exaggerate immunological responses to pregnancy. Alternatively, other TNFα-related processes such as regulation of angiogenesis may be involved. To discriminate between these, future measurements of other inflammatory markers as well as growth factors are planned. (NIH HL079647 and HL 14985).

ENDOTHELIAL NITRIC OXIDE SYNTHASE PHOSPHORYLATION RESPONSES TO BASIC FIBROBLASTIC GROWTH FACTOR AND ESTROGEN IN FOLLICULAR, LUTEAL AND PREGNANT DERIVED OVINE UTERINE ARTERY ENDOTHELIAL CELLS

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Objectives: Compared to the ovine luteal phase, uterine blood flow and estrogen concentrations are elevated during the follicular phase but even more so during pregnancy. Uterine blood flow is partially regulated by uterine artery endothelium via endothelial nitric oxide synthase (eNOS) activation, which produces the potent vasodilator nitric oxide (NO). NO acts in a paracrine fashion to cause vasorelaxation of uterine artery vascular smooth muscle. Basic fibroblast growth factor (bFGF) and estrogen are vasoactive mediators that may alter NO production. bFGF has been shown to relax isolated arteries, and in endothelial cultures bFGF increases NO production and eNOS expression. It is unclear if bFGF and estrogen activate eNOS by phosphorylation through either the PI3 Kinase (Akt) or MAPK (ERK 1/2) pathway. We therefore studied the phosphorylation of Akt and ERK1/2 in response to bFGF and estrogen to determine if there was an association with eNOS phosphorylation. **Methods:** Uterine Artery Endothelial Cells (UAEC) from Luteal (Lut), Follicular (Fol) and Pregnancy (Preg) sheep were cultured (Passage 4–5, n = 3 each phase) in the presence or absence of estrogen (10 nM) for 48 hr. At 80% confluence cells were serum starved (4 hr) and subjected to: Control or bFGF (10 ng/ml) for 10 min. Phosphorylation of Akt, ERK 1/2 and Ser635-eNOS were evaluated by western analysis and normalized to the total amount of eachrespective protein. Data are expressed as mean fold of control ± SEM. **Results:** The Akt pathway was activated in response to estrogen for Fol (1.27 ± 0.38) and Lut (2.89 ± 0.48), but not Preg (0.74 ± 0.31) UAECs. With bFGF alone the Fol (0.83 ± 0.08) and Preg (1.01 ± 0.52) did not increase Akt, whereas the Lut (2.03 ± 0.67) cells showed robust rises. When both estrogen and bFGF were added there was an increased Akt phosphorylation for Fol (2.47 ± 1.83), Lut (1.89 ± 1.09) and Preg (3.34 ± 2.8). The ERK1/2 phosphorylation response to estrogen was greatest for Fol (ERK1: 2.8 ± 2.42 & ERK2: 2.6 ± 1.6). For Lut and Preg UAECs there was no noticeable increase in either ERK1 or 2 phosphorylation. Addition of bFGF increased Fol (13.6 ± 10.4), Lut (9.01 ± 2.84) and Preg (3.89 ± 1.98) ERK1 phosphorylation with similar responses for ERK2. These effects appeared the same when both estrogen and bFGF were present. eNOS phosphorylation in response to estrogen was greatest for Lut (1.95 ± 1.2) and Fol (1.58 ± 0.52) with no change in Preg (0.74 ± 0.31). With bFGF the same eNOS phosphorylation pattern occurs with Lut (1.95 ± 1.21), Fol (1.36 ± 0.27) and Preg (0.36 ± 0.17). With bFGF in estrogen treated UAECs, eNOS phosphorylation was noted in Lut (1.26 ± 0.7), but not Fol (0.95 ± 0.28) or Preg (0.72 ± 0.15) UAECs. **Conclusions:** bFGF appears to activate the cell through the ERK1/2 pathway for UAEC from all three physiologic groups. Estrogen appears to activate the Akt pathway in follicular and luteal UAEC and this mirrors the phosphorylation of eNOS at Serine 635.

ANCESTRY-ASSOCIATED VARIATION OF ENDOGENOUS ANTIOXIDANT CAPACITY DURING PREGNANCY AT HIGH ALTITUDE

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The mechanisms by which high-altitude ancestry protects against hypoxia-associated maternal vascular dysfunction during pregnancy and fetal growth restriction are unclear. We considered that hypoxia-induced oxidative stress contributes to the increased incidence of these disorders at altitude and that the protection afforded by high-altitude Ancestry may be due, in part, to greater antioxidant activity.

Objective: To determine the effects of altitude, ancestry and pregnancy on endogenous antioxidant activity (i.e. superoxide dismutase (SOD) and catalase (CAT)). **Methods:** Maternal erythrocyte CAT and SOD activity were measured across pregnancy (20 and 36 weeks) and in the non-pregnant state (NP) in Andean and European women at low (416 m, Santa Cruz, Bolivia) or high (3600–4100 m, La Paz or El Alto, Bolivia). CAT activity was assessed via the spectrophotometric method of Beers and Sizer. SOD activity was determined by the xanthine oxidase/xanthine/cytochrome *c* method of McCord and Fridovich. **Results:** There was no difference in CAT or SOD activity between ancestry groups at low altitude. Pregnancy decreased CAT activity in Andeans (23% and 26% lower at 20 w and 36 w vs.. NP; $p < 0.01$ each) and Europeans (24% and 21% lower at 20 w and 36 w vs.. NP; $p < 0.001$ each) at low altitude. Pregnancy did not alter SOD activity in either ancestry group. At high altitude CAT activity was greater in Andean than European women at each time point (NP, $p < 0.05$; 20 w, <0.01; 36 w, $p < 0.001$) but was unaffected by pregnancy. Similarly, SOD activity was elevated in Andean ($p < 0.001$) but not European ($p = NS$) women during pregnancy at high altitude, and was greater in Andeans than Europeans at 36 w ($p < 0.001$). **Conclusions:** Our findings are consistent with the possibility that elevated endogenous antioxidant activity contributes to the protection against hypoxia-associated maternal vascular dysfunction during pregnancy and reduced fetal growth seen in high-altitude Andean populations.

eNOS AUGMENTS UTERO-PLACENTAL BLOOD FLOW AND UTERINE AND SPIRAL ARTERIAL REMODELING DURING PREGNANCY IN MICE

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Objectives: In mice lacking the eNOS gene, the normal increases in maternal cardiac output and enlargement of the heart and aorta during pregnancy are blunted, and fetal body weight is reduced (−17%). eNOS is expressed in the uterine, and utero-placental vasculatures, therefore we hypothesized that their remodeling would also be blunted in pregnant eNOS −/− mice, leading to an elevated utero-placental vascular resistance and decreased blood flow which in turns leads to increased hypoxia in the placenta contributing to fetal growth restriction. **Methods:** Using an ultrasound biomicroscope, utero- and umbilical-placental blood velocity waveforms and umbilical arterial diameters were determined in pregnant control (C57B1/6J) and eNOS −/− mice (N = 5–6 mothers) under light isoflurane anesthesia at late gestation (E17.5). Spiral artery morphology and uterine arterial diameters were evaluated from vascular corrosion casts. Peak systolic (PSV) and end-diastolic velocities (EDV) were used to calculate Resistance Index (RI = (PSV − EDV)/PSV). Hypoxyprobe-1 immunohistochemistry was used to identify hypoxic regions in the placenta. **Results:** Calculated uterine blood flow normalized to the weight of the uterus and its contents was significantly reduced in eNOS −/− mothers (2.7 (SE) ± 0.9 ml/min/100 g) relative to controls (6.0 ± 0.4 ml/min/100 g). This was caused by significant reductions in uterine arterial diameter (0.16 ± 0.01 mm vs. 0.24 ± 0.01 mm in controls) and uterine arterial mean blood velocity (196 ± 23 mm/s vs. 230 ± 19 mm/s in controls). In addition, Resistance Index of the uterine artery was significantly elevated in the pregnant eNOS −/− mice (0.70 ± 0.02 vs. 0.46 ± 0.01 in controls). Corrosion casts indicated reduced spiral arterial coiling in eNOS −/− mothers relative to controls. Strong immunoreactivity was detected in the spongiotrophoblast and trophoblast giant cell layers of the junctional zone of eNOS −/− placentas, whereas fainter staining was only detected in the spongiotrophoblast cell layer in controls. In the umbilical circulation, there were significant reductions in mean blood velocity (39 ± 3 mm/s vs. 46 ± 2 mm/s in controls) and arterial diameter (0.48 ± 0.01 mm vs. 0.52 ± 0.01 mm in controls) however blood flow normalized to fetal weight was not significantly different between the groups, and Resistance Index was only slightly higher (0.93 ± 0.01) than controls (0.90 ± 0.01). **Conclusions:** These findings suggest eNOS plays an important role in uterine and spiral artery remodeling, and in augmenting utero-placental blood flow during pregnancy. In its absence, there was apparent placental hypoxia in the junctional zone of the placenta. Thus, placental hypoxia may contribute to fetal growth restriction in eNOS −/− mice.

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INFLUENCE OF MATERNAL NUTRITION ON mRNA EXPRESSION OF ANGIOGENIC FACTORS AND RECEPTORS IN MATERNAL AND FETAL JEJUNUM

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Objectives: Objectives were to examine effects of nutrient restriction and dietary Se on mRNA expression of major angiogenic factors and their receptors in maternal jejunal mucosal scrape and fetal jejunum tissue of primigravid sheep and their offspring. **Methods:** Targhee-cross ewe lambs (n = 36) were allotted randomly to one of four treatments in a 2 × 2 factorial arrangement. Treatments were plane of nutrition (control [CON; 100% of requirements] vs. restricted [RES; 60% of controls]) and dietary Se (adequate Se [ASe; 6 μg/kg BW] vs. high Se [HSe; 80 μg/kg BW]) provided as Se-enriched yeast. Selenium treatments were initiated 21 d prior to breeding and restriction treatments on d 64 of gestation. Nutrient requirements were based on ewe BW, and nutrient intake was adjusted every 2 weeks to account for changes in BW. Tissues were harvested on d 135 ± 5 of gestation and analyzed with real time RT-PCR to determine mRNA expression of major angiogenic factors and their receptors. **Results:** As reported previously, nutrient restriction reduced maternal jejunal mass, vascular density, and capillary area. In addition, nutrient restriction reduced fetal BW and small intestine mass. Nutrient restriction upregulated mRNA expression of vascular endothelial growth factor (*VEGF;* $P < 0.01$), kinase insert domain receptor (*KDR;* $P = 0.03$), vascular endothelial growth factor/vascular permeability factor receptor (*FLT1;* $P = 0.02$), neuropilin 1 and 2 ($P = 0.07$ and 0.03, respectively) in maternal mucosal scrape. Basic fibroblast growth factor was lowest in RES-ASe and CON-HSe and greatest in RES-HSe and CON-ASe ($P < 0.01$). A nutrition × Se interaction ($P = 0.02$) in hypoxia inducible factor-1α upregulated mRNA expression for RES-HSe over all other treatments. Endothelial tyrosine kinase (*TIE2*), an angiopoietin- 1 receptor was lowest ($P \leq 0.06$) in CON-HSe compared to all other treatments. Fetal jejunal tissue mRNA expression of soluble guanylate cyclase was downregulated ($P = 0.01$) with nutrient restriction. Supplementation of Se upregulated *TIE2* mRNA expression ($P = 0.10$). Nutrition × Se interaction was observed for angiopoietin-2 where CON-HSe and RES-ASe were greatest, CON-ASe intermediate and RES-HSe lowest ($P = 0.01$). Reductions in maternal jejunal mass and vascular density result in hypoxia that likely stimulates expression of *VEGF* and receptors *FLT* and *KDR.* **Conclusions:** These maternal changes provide insight into underlying mechanisms associated with reduced fetal BW at d 135 of gestation. Our laboratory is continuing research efforts to understand the impact of these responses in developmental programming.

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EFFECTS OF ESTROGEN ON PRESSURE-LENGTH-DIAMETER RELATIONSHIPS IN UTERINE ARTERIES DURING OVINE PREGNANCY

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Introduction: Remodeling of the uterine vasculature is a hallmark of normal human and ovine pregnancy and is directly associated with fetal growth during the third trimester. By contrast, insufficient vascular remodeling during pregnancy is associated with low birth weights and, as described by the Barker Hypothesis, potentially with Developmental Origins of Adult Onset Cardiovascular Disease. Defects in uterine artery mechanical properties (i.e., pressure-diameter and pressure-length relationships) may be partly responsible. In addition, as a primary placental-derived hormone of pregnancy, estrogen is likely to be a significant contributor to remodeling and uterine artery mechanical properties. **Objective:** To quantify the effects of endogenous estrogen on uterine large artery mechanical properties during pregnancy. **Methods:** Placental-derived estrogen in the ovine pregnant model was removed using aromatase inhibitor (CGS 20267; Letrozole; 20 mg loading dose IM then daily 125–150 mg/kg) over a two week period prior to sacrifice during the third trimester (n = 4 animals) and compared to vehicle treated pregnant animals at the same gestational age (n = 4). Intact branching segments of mesometrial uterine arteries (2–6 mm outer diameter at dissection) were transmurally pressurized in stepwise static (no flow) fashion in an isotonic bath solution, allowing unrestricted longitudinal and circumferential expansion. Artery segment length and diameter were measured at each pressure step to create curves from which longitudinal and circumferential stiffness were calculated. **Results:** Letrozole treatments did not significantly alter the longitudinal elastic modulus E_{long} or the circumferential elastic modulus E_{circ} of primary, secondary or tertiary arteries in the uterine arterial network. However, in all cases, E_{long} (Mean ± SE; 433 ± 50 kPa) was substantially greater (3.8 ± 0.5 fold) than E_{circ} (120 ± 15 kPa) ($P < 0.0001$). **Implications:** These data apply only to the effects of endogenous estrogen production on large artery remodeling in the late stages of pregnancy under static no-flow conditions. The nearly 4-fold difference between longitudinal and circumferential stiffness suggests that for this range of *ex vivo* increases in pressure, uterine arteries dilate more readily than they lengthen. A more quantitative understanding of the changes in uterine artery moduli during normal pregnancy may yield insight into factors that control vascular network growth and development. Furthermore, since arterial mechanics strongly influence blood flow patterns, understanding these mechanics may improve our knowledge of hemodynamic adaptation to normal pregnancy and provide clues to the dysfunction of complicated pregnancies, such as Preeclampsia.

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EFFECTS OF MATERNAL DIET ON CIRCULATING HORMONE CONCENTRATIONS DURING MID- TO LATE PREGNANCY IN FIRST-PARITY EWES

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Objective: This study was designed to examine the combined effects of supranutritional selenium (Se) and global levels of nutrition on maternal metabolic hormones and progesterone during pregnancy. **Methods:** Primiparous Rambouillet ewes (n = 84) were allotted randomly to one of six treatments in a 2 × 3 factorial design. Selenium treatments [Adequate Se (ASe, 7.4 μg/kg BW) vs. High Se (HSe, 85 μg/kg BW] were initiated at breeding, and global nutritional level [control (C, 100% of requirements) vs. restricted (R, 60% of C) vs. overfed (O, 140% of C)] was initiated on d 50 of pregnancy. Beginning on d 50 and through parturition, jugular blood samples were collected every 2 weeks, and triiodothyronine (T3), thyroxine (T4), insulin (INS), and progesterone (P4) concentrations were determined. At term, mammary gland wt and lamb birth wt were determined. **Results:** Although Se did not impact maternal hormone or metabolic status, global nutrition influenced T3, T4, INS, and progesterone. On d 64, R ewes had decreased ($P < 0.01$) T3 and T4 compared to C and O ewes. By d 78, and through the remainder of pregnancy, O ewes had greater ($P < 0.01$) concentrations of T3 and T4 compared to C ewes, which were greater than ($P < 0.01$) those of R ewes for the remainder of pregnancy. While there was no difference in INS concentrations between R and C ewes throughout pregnancy, by d 134 O ewes had increased ($P < 0.01$) INS concentrations compared to R and C ewes. Progesterone concentrations on d 64 and 78 were elevated ($P < 0.01$) in R ewes compared to C and O ewes, which did not differ. From d 92 to term, progesterone concentrations were decreased ($P < 0.01$) as global level of maternal nutrition increased (i.e., R > C > O ewes). Selenium did not impact mammary gland wt at term or lamb birth wt. However, lamb birth wt in O and R ewes was reduced ($P < 0.05$) compared to C ewes (4.2 and 4.0 vs. 4.6 ± 0.1 kg). Mammary gland wt (g/kg maternal wt) was reduced in O versus R and C ewes, which did not differ (12.9 vs. 15.7 and 16.0 ± 1.0 g/kg). **Conclusions:** Although Se is known to influence thyroid hormone metabolism, supranutritional levels during pregnancy did not alter circulating T3 and T4 concentrations. Alterations in maternal endocrine status may have influenced maternal transport of nutrients to the developing fetus, resulting in the observed fetal growth restriction. Further, mammary gland wt may have been impacted by maternal endocrine patterns during pregnancy. These alterations in mammary gland wt may impact mammary gland function and colostrum production, thereby further impairing growth of developing neonates.

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ATTENUATED UTERINE BLOOD FLOW IS AN EARLY DEFECT IN GROWTH-RESTRICTED PREGNANCIES INDUCED BY OVERNOURISHING ADOLESCENT DAMS

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Uterine blood flow (UtBF) is a major regulator of transplacental fetal nutrient supply and cross sectional studies of normal sheep pregnancies indicate a two to three fold increase in UtBF during the second half of gestation.

Objective: The aim of this study was to serially measure uterine blood flow from mid to late pregnancy in a paradigm of relatively late onset placental and fetal growth restriction. **Methods:** Singleton bearing adolescent dams were fed high (H) or control (C) nutrient intakes to induce putatively compromised or normal pregnancies, respectively. A Transonic flow probe was attached to the uterine artery of the gravid horn on Day 83 of gestation and following a 5 day recovery period, UtBF was recorded continuously for 2 hours, three times weekly until ~Day 135, when pregnancies were either terminated ($n = 4$ per group) or ewes allowed to spontaneously deliver at term ($n = 5$ or 6 per group). Pregnancy outcome was similarly determined at term in contemporaneous ewes without UtBF assessment (H, $n = 15$ and C, $n = 13$). **Results:** Placental and fetal weights were lower ($P < 0.001$) in H compared with C intake groups and were independent of flow probe surgery and monitoring. Fetal weights (adjusted to Day 145 of gestation) for ewes with flow probes were 3964 ± 316 and 5361 ± 127g for H and C groups, respectively ($P < 0.001$). Placentome weight at autopsy and fetal cotyledon weight at term were reduced in H relative to C groups by 30 and 50%, respectively. Full blood flow data were obtained for 17 of 19 pregnancies, while partial data were collected to Day 109 and 121 respectively for the two high intake dams with the lowest initial flows. Uterine blood flow was lower in H compared with C groups at Day 88 of gestation (196 ± 23.1 vs. 337 ± 19.4 ml/min, $P < 0.001$) and was positively correlated with adjusted fetal weight at term, irrespective of treatment group ($r = 0.581$, $P < 0.01$). UtBF increased throughout the second half of gestation in both groups and at Day 135 was on average 2.2(H) and 1.9(C) fold higher than on Day 88. Linear regression analysis of UtBF against day of gestation revealed that the mean slope was equivalent (5.5 vs. 5.3 ml/min/day) and the mean intercept lower (212 vs. 370 ml/min, $P < 0.001$) in H compared with C groups, respectively. **Conclusion:** This study confirms previously reported increases in UtBF over time in normal pregnancies and demonstrates the feasibility of serially measuring UtBF within the same individuals for a protracted period during the second half of gestation. The lower UtBF at the initial assessment in putatively compromised pregnancies, ahead of any reduction in placental or fetal weight, is commensurate with previously reported decreases in placental angiogenic growth factor expression at mid-gestation, and, suggests that attenuated UtBF is an early defect in this nutritionally- mediated model of feto-placental growth restriction. It remains to be established whether manipulating uterine blood flow could help to prevent fetal growth restriction in this adolescent paradigm.

WIDE VARIATIONS IN GESTATIONAL DIETARY INTAKE DIFFERENTIALLY IMPACT ON PREGNANCY OUTCOME IN YOUNG ADOLESCENTS AND INFLUENCE EARLY POSTNATAL OFFSPRING PHENOTYPE

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The risks of miscarriage, premature delivery and low birth weight are particularly acute in young adolescent girls who are still growing at the time of conception. Separate studies using ovine paradigms suggest that nutrient intakes at both ends of the nutritional spectrum differentially impact on fetal nutrient supply and result in fetuses with contrasting body composition in late gestation.

Objective: The aim here was to determine the impact of these wide variations in gestational intake on pregnancy outcome after spontaneous delivery at term and characterise the early phenotype of the resulting offspring. **Methods:** Adolescents of equivalent age, weight and adiposity were implanted with a single embryo derived from a single sire on day 4 post-estrus. Thereafter, ewes were either offered a moderate intake to maintain maternal adiposity throughout gestation (optimally nourished control [C], $n = 18$), undernourished to maintain weight at conception but deplete maternal body reserves (UN, $0.75 \times C$ intake, $n = 23$), or overnourished to promote rapid maternal growth and increased adiposity (ON, $2.25 \times C$, $n = 22$). **Results:** For C, UN and ON dams, respectively, the gestational change in weight was +8.3, −0.7 and +32.9 kg while the change in external adiposity score was 0, −0.7 and +1.3 units. Gestation length (days) was equivalent in C and UN dams (147.3 and 147.5) and longer than in ON dams (143, $P < 0.00001$). Lamb birth weight was influenced by pregnancy intake ($P < 0.001$) and was lowest in ON (4.37 kg), intermediate in UN (5.05 kg) and highest in C (5.56 kg) groups. Fetal cotyledon mass was similar in C and UN groups (167 and 157 g) and reduced in the ON group (92 g, $P < 0.001$). Thus, reduced placental size limits fetal growth in the ON dams while the more modest reduction in fetal weight in UN dams was due to reduced nutrient availability in the maternal circulation. Colostrum yield at parturition was equivalent in UN and ON dams but lower ($P < 0.001$) than in C, total colostrum IgG and energy content were attenuated ($P < 0.02$) relative to C. All lambs received 50ml colostrum/kg Bwt. (own dam or colostrum bank) at birth and after parturition all ewes were nourished to maximise milk yield. Lamb hematocrit at birth was lower ($P < 0.005$) in UN compared with ON and C groups, but was equivalent at weaning. Neonatal fractional growth rate (FGR) to 42 days, and to weaning at 77 days of age, was equivalent in C and UN offspring and lower than in ON offspring (6.89, 7.34 and 8.93 %/day at 77 days, respectively, $P = 0.00001$). Similarly, both total and LDL cholesterol at birth were elevated in the low birth offspring of ON dams relative to both the UN ($P < 0.005$) and C ($P < 0.05$) groups. This difference remained significant throughout lactation for the ON vs. UN comparison only, ($P < 0.001$). **Conclusion:** This study demonstrates contrasting routes to prenatal growth restriction in young adolescent mothers, which differentially impact on pregnancy outcome and on early postnatal growth and lipidemia.

EXPLORING GENE CANDIDATES FOR NATURAL SELECTION IN HIGH-ALTITUDE PREGNANCY

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Fetal growth is slowed at high altitude (>2500 m) and preeclampsia more common, both of which decrease birth weight and raise perinatal morbidity/mortality. We considered that (1) natural selection at high altitude would have targeted genetic factors contributing to these disorders and (2) the genes involved likely included those in the hypoxia-inducible factor (HIF) pathway.

Objectives: To test whether genetic adaptations in HIF-regulatory or targeted genes had been targeted, we compared single nucleotide polymorphisms (SNPs) in Andeans *vs.* low-altitude control populations (low-altitude Amerindians and Han Chinese). **Methods:** In 50 multi-generational high-altitude Andeans, 593 SNPs were evaluated in 59 HIF-pathway genes. Results were analyzed using locus specific branch lengths (LSBL) and the natural log of the ratio of heterozygosity (lnRH) with a sliding windows approach, in which reduced heterozygosity suggests directional selection. Regions that fell in the 0.05 tail of respective negative (lnRH) or positive (LSBL) empirical distributions were considered significant. **Results:** LSBL and lnRH assessments converged in identifying three gene regions as differing between Andeans and controls: inducible nitric oxide synthase, tenascin-C, and AMP kinase alpha-1 (*syn.* AMPKα-1). Each is involved in pregnancy and hypoxia-related vascular remodeling. LnRH results identified 2804 regions that differed between Andeans and controls, for which a high (31%) fraction were within HIF-related gene regions. **Conclusions:** The high proportion of HIF-related SNPs within low heterozygosity regions supports the involvement of HIF pathway genes in hypoxia-related adaptations in the Andean population. The functional roles of the three candidate genes suggest the mechanism of Andean adaptation during pregnancy targets vascular remodeling. (NIH HL60131, TW 01188, HL07171; NSF Graduate Research Fellowship).

EVIDENCE THAT THE SNAT4 ISOFORM OF SYSTEM A IS FUNCTIONAL IN HUMAN PLACENTAL MICROVILLOUS MEMBRANE

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Introduction: The system A amino acid transporter (comprising SNAT1, SNAT2 and SNAT4 isoforms) is important for neutral amino acid transfer across the placenta. Reduced placental system A activity is associated with fetal growth restriction (FGR) in humans and data from knock-out mice studies suggest that placental SNAT4 is implicated in fetal growth. We have shown the SNAT4 isoform is expressed in human placenta during first trimester (FT) and at term, along with SNAT1 and SNAT2. Furthermore, SNAT4 protein is localized to the syncytiotrophoblast suggesting a role in amino acid transport from mother to fetus. System A activity increases between FT and term, coinciding with increased fetal nutrient demands. Our data have shown that SNAT4 protein expression increases between FT and term suggesting this isoform has an important role for amino acid transport in relation to fetal growth. Previous investigations of placental system A activity have used substrates transported by all three isoforms. Therefore the contribution from each isoform to amino acid transport by system A in the placenta has yet to be determined. **Objectives:** To develop an experimental paradigm to test the hypothesis that SNAT4 is functional in human placenta. **Methods:** System A is sodium-dependent and transports small neutral amino acids and the amino acid analogue MeAIB, used as a specific substrate for this transporter. According to single oocyte injection studies, SNAT4 has a unique ability to also transport cationic amino acids. We therefore investigated arginine-inhibitable ^{14}C-MeAIB uptake by microvillous membrane (MVM) vesicles isolated from FT ($n = 3$) and term ($n = 6$) placenta over a 1 minute time course. Experimental conditions were optimized to promote uptake by SNAT4 (extravesicular buffer pH8.4 and an inwardly directed sodium gradient of 90 mM). The arginine-inhibitable component of uptake provides an estimate of SNAT4 contribution to total system A activity. **Results:** Total system A activity, measured at 30 seconds (initial rate), was higher in term MVM compared to FT ($p < 0.05$, unpaired t-test). MeAIB uptake by term MVM vesicles was significantly inhibited by 20 mM and 30 mM arginine ($p < 0.01$ and $p < 0.0001$ respectively, 2-way ANOVA). The arginine (30 mM) inhibitable component of uptake at 30 seconds was $32.9\% \pm 2.7$. 30 mM arginine also significantly inhibited MeAIB uptake by FT MVM vesicles ($p < 0.001$, 2-way ANOVA). Intravesicular volume was unaffected by 30 mM arginine confirming that the inhibitory effects were not attributable to osmolality-induced changes in volume. **Conclusions:** The cationic amino acid arginine inhibits MeAIB uptake by FT and term MVM vesicles. We propose that arginine-inhibitable MeAIB uptake represents SNAT4 activity. These data suggest that under the conditions of the assay, SNAT4 contributes to a third of total system A activity in term MVM. Changes in the activity of this isoform may contribute to reduced placental system A activity associated with human FGR.

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PARATHYROID HORMONE (PTH)/PARATHYROID HORMONE RELATED PROTEIN (PTHRP) TYPE 1 RECEPTOR IN THE PLACENTA OF PTHRP KNOCKOUT MICE AND HUMAN PLACENTA

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Objectives: To determine whether the protein expression of PTH/PTHrP type 1 receptor (PTHR1) is altered in the placentas of mice in which the PTHrP gene is ablated (nulls) compared with their wild type (+/+) and heterozygous (+/−) siblings and to localise this receptor within both mouse and human placentas. **Introduction:** Both PTH and PTHrP are ligands that bind to PTHR1, a G- protein coupled receptor, and play important roles in regulating transport of calcium across the placenta and fetal calcium homeostasis. In PTHrP knockout (−/−) fetuses the balance of these two ligands is altered, with circulating levels of PTH being significantly increased compared with their (+/+) and (+/−) siblings, whilst fetal PTHrP is markedly diminished. The impact of this on the expression of PTHR1 remains ill-defined. Similarly, the localisation of PTHR1 within the placenta remains unclear, although previous evidence of PTH binding to both microvillous (MVM) and basal (BM) membranes of human placenta suggest that PTHR1 is localised to both syncytiotrophoblast plasma membranes. Here we examine this issue using Western blotting (WB) and immunohistochemistry (IHC) with mouse kidney (K) as positive control. **Methods:** PTHR1 protein expression was examined by WB in plasma membrane fractions from placentas of mice comprising litters of (+/+), (+/−) and (−/−) fetuses, and localisation examined by IHC. PTHR1 expression was also compared in four matched pairs of MVM and BM isolated from individual human placentas at term. **Results:** A major immunoreactive species of ~45 kDa was detected in mouse placenta compared with ~60 kDa in K, perhaps reflecting different glycosylation patterns. Semi-quantitative analysis by densitometry (arbitrary density units; mean ± SEM) of 3 litters revealed there was no significant difference in PTHR1 expression between placentas of (−/−) fetuses (4.39 ± 0.34) compared with their (+/+) (2.47 ± 0.06) and (+/−) (4.37 ± 1.15) siblings. PTHR1 staining of mouse placenta appeared localised to trophoblast. MVM PTHR1 appeared as a single band of 60kDa which co-migrated with signal in K, whilst in BM there was a doublet of 57 and 60kDa repectively, of similar signal intensity. PTHR1 expression demonstrated significantly higher expression in MVM (7.21 ± 1.71) compared with BM (2.47 ± 0.27; $P < 0.05$ paired 't' test). β-actin expression confirmed comparable protein loadings between groups. **Conclusions:** These data suggest that PTHR1 expression in PTHrP (−/−) fetuses is unaltered by fetal PTHrP or PTH concentrations and is localised to mouse trophoblast plasma membranes. We have demonstrated that PTHR1 is present in both MVM and BM, with differential expression of PTHR1 moieties apparent at these two loci. However, distinct signalling pathways for this receptor are implicated as adenylate cyclase (involved in PKA activation) is polarised solely to BM.

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EXPRESSION AND ACTIVTY OF HENT1 AND HENT2 ARE REGULATED BY ADENOSINE RECEPTORS IN PLACENTA MICROVASCULAR ENDOTHELIUM FROM PREECLAMPSIA

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Objectives: Preeclampsia is associated with low equilibrative nucleoside transporter 1 (hENT1), but high hENT2 expression and activity in human placental microvascular endothelium (hPMEC) compared with cells from normal pregnancies. We studied whether these changes are associated with adenosine receptor A_{2A} activation. **Methods:** Experiments were performed in hPMEC isolated from normal ($n = 10$) and preeclamptic pregnancies ($n = 5$). Adenosine concentration in culture medium was quantified by h.p.l.c. [^{3}H]Adenosine uptake (10 μM, 4 μCi/ml, 20 s, 22°C) was measured in absence or presence of nitrobenzylmercaptopurine riboside (NBTI, 1 μM, ENT1 inhibitor) and/or hypoxanthine (2 mM, ENT2 substrate). Role of A_{2A} adenosine receptors was evaluated using the selective agonist CGS-21680 (30 nM) and the antagonist ZM-241385 (100 nM). Protein abundance was detected by Western blot. **Results:** Adenosine concentration in culture medium was higher in preeclampsia compared with normal pregnancy (2.2 vs 0.96 pM, $P < 0.05$, unpaired Student t test). hENT1-adenosine uptake and protein abundance were reduced (~90 and 95%, respectively) in preeclampsia. CGS-21680 reduced hENT1-adenosine uptake in normal pregnancies (~21%) without changing protein abundance; however, this agonist did not alter hENT1-adenosine transport in preeclampsia. ZM-241385 by itself increased hENT1-adenosine transport (~7-fold) and protein abundance (~2-fold) and reversed the CGS-21680 inhibition of hENT1-adenosine transport in normal cells. In preeclampsia ZM-241385 increased hENT1-adenosine transport over the basal value (~3 fold) detected in normal pregnancies, but did not alter protein abundance. Preeclampsia increased hENT2-adenosine transport (~5-fold) and protein abundance (~2-fold) compared with normal pregnancies. CGS-21680 or ZM-241385 reduced the stimulatory effect of preeclampsia on hENT2-adenosine transport (~57 and ~65%, respectively) and protein abundance (~23 and ~85%, respectively), but did not alter transport in cells from normal pregnancies. CGS-21680 and ZM-241385 effects were accumulative on transport and protein abundance in preeclampsia. However, only hENT2 protein abundance was reduced (~29%) by ZM-241385 in normal pregnancies. **Conclusions:** Adenosine via A_{2A} receptor could be responsible of the reduced hENT1 expression and activity in preeclampsia. Moreover, a compensatory increase in expression and activity of hENT2 in preeclampsia may be associated with a different adenosine receptor sensible to ZM-241385 in hPMEC.

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THE EFFECT OF FGF-4 ON CYTOTROPHOBLAST DIFFERENTIATION

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Objectives: Our previous work has suggested that two separate populations of cytotrophoblast exist in the first trimester placenta, one in cell islands in anchoring villous tips that are committed to EVT differentiation (which we have previously termed EVT progenitors), and the other that exist in a monolayer underlying the syncytiotrophoblast and are committed to syncytiotrophoblast differentiation. Furthermore, we have exploited the extended viability of EVT progenitors in culture to isolate and study this population. However, conflicting evidence has been presented which suggests that in the first trimester villous cytotrophoblasts are bipotential and capable of differentiating into either extravillous trophoblasts (EVTs) or syncytiotrophoblast, and that this is able to be directed towards the EVT pathway by FGF-4. Therefore, we have investigated the effect of FGF-4 on EVT progenitor differentiation. **Methods:** 680 villous explants from 6 first trimester placentae were cultured with or without exogenous FGF-4 and the frequency of EVT outgrowth in each explant culture was determined. EVT progenitors were isolated from additional first trimester villous explants. The expression of FGFR-2 and HLA-G by EVT progenitors was examined by immunohistochemistry. **Results:** FGF-4 did not significantly alter the frequency of EVT outgrowths from villous explants, which were only observed from anchoring villous tips. EVT progenitors isolated from first trimester placentae expressed FGFR-2. However, FGF-4 did not alter the proportion of EVT progenitors that expressed HLA-G after 4 days in culture. **Conclusions:** FGF-4 did not increase the frequency of EVT outgrowth from villous explants or affect the differentiation of EVT progenitors into EVT.

REGULATION OF PLACENTAL LIPOPROTEIN LIPASE ACTIVITY IN CULTURED HUMAN TROPHOBLAST CELLS

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Objectives: To ensure optimal fetal development adequate placental transport of fatty acids is crucial. Essential and polyunsaturated fatty acids are important for the function and structure of cell membranes, as precursors for cellular signaling molecules, and are critical for the development of the brain and retina. During late pregnancy the fetal demand for fatty acids increases, to ensure fetal brain development and to support fat deposition. Intact triglycerides (TGs) cannot be directly transferred across the placenta. Triacylglycerol hydrolases in the microvillous membrane (MVM) of the syncytiotrophoblast hydrolyze maternal TGs, enabling placental transfer of free fatty acids (FFAs). Lipoprotein lipase (LPL) is one such hydrolase found in the MVM of the human placenta. The activity of LPL has been reported to be decreased in placentas from growth-restricted fetuses, and up-regulated in placentas from large-for-gestational-age babies of mothers with insulin-dependent diabetes. Factors responsible for the regulation of placental LPL are not well established. In the current study, we hypothesized that cytokines and estradiol stimulate placental LPL activity. **Methods:** Cytotrophoblast cells were isolated from healthy, term placentas and cultured for 66 hours to allow for differentiation. The tested effectors, 17β-estradiol, IL-6, TNF-α, or insulin, were added to the cell culture media and incubated with the trophoblast cells for 3 or 24 hours at 37°C. LPL activity was measured as the capacity to hydrolyze triglycerides (^{3}H-labelled trioleate) to FFA (^{3}H-labelled oleic acid) at pH 8 (optimum for LPL) and in the presence of fetal calf serum (to provide apoC2, a cofactor for LPL). **Results:** Trophoblast LPL activity was up-regulated in cells treated with 0.2 μg/ml IL-6 ($n = 7$, $p < 0.05$) while TNF-α ($n = 8$) had no effect. 17β-estradiol (100 ng/ml, for 3 hours) increased LPL activity by 55%, however these changes did not reach statistical significance ($n = 4$, $p = 0.056$). Insulin did not alter LPL activity in cultured trophoblast cells. **Conclusion:** Insulin is known to stimulate LPL activity in adipose tissue, however we found placental LPL to be unresponsive to insulin under normoglycemic conditions. These findings are in line with our previous studies using isolated villous fragments showing no effect of insulin in normoglycemic media. Fetuses require a constant supply of fatty acids, independent of maternal feeding, suggesting a unique regulation of placental lipid metabolism and transfer compared to other cell types. Estradiol is known to stimulate LPL and this corresponds nicely with estrogen mediated increases in maternal lipoproteins in late pregnancy. Higher maternal body mass index (BMI) is strongly correlated to birth weight and we found that IL-6, a cytokine known to be elevated in overweight women, increases the activity of placental LPL. This would potentially increase the level of FFAs available for transfer to the fetus. This finding may in part explain the increased birth weight associated with high maternal BMI.

THE ISOLATION AND CHARACTERISATION OF EXTRAVILLOUS TROPHOBLAST PROGENITORS FROM FIRST TRIMESTER PLACENTAL EXPLANTS

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Objectives: It is widely accepted that most if not all villous cytotrophoblasts from term placentae are committed to differentiate into syncytiotrophoblast, but that early in gestation villous cytotrophoblasts are bipotential and capable of differentiating into either extravillous trophoblasts (EVTs) or syncytiotrophoblast. However, the factors that direct cytotrophoblast differentiation down these different lineages in the first trimester remain unclear. In contrast, our previous work has suggested that two separate populations of cytotrophoblast exist in the first trimester, one committed to EVT differentiation (which we have previously termed EVT progenitors), and the other to syncytiotrophoblast differentiation. In this work we have exploited the extended viability of EVT progenitors in order to isolate and characterise this population. **Methods:** First trimester villous explants were cultured for 10 days then subjected to sequential trypsinization. Viable cells that adhered to Matrigel following trypsinization were cultured for up to 5 days and characterised by immunohistochemistry. **Results:** A viable population of >90% trophoblasts were obtained. These putative EVT progenitors proliferated in culture and expressed markers characteristic of EVT progenitors including $\alpha v\beta 6$ integrin and CD9. Over 5 days of culture putative EVT progenitors did not syncytialise, but rather approximately 20% differentiated into HLA-G positive EVTs. **Conclusions:** It is likely that the isolated putative EVT progenitors are the population of EVT progenitors previously identified *in vivo*. The characteristics of these isolated putative EVT progenitors provide further evidence for separate progenitors of EVT and syncytiotrophoblast in the first trimester.

COMPARTMENTALIZING VEGF-ACTIVATED ERK2/1 PATHWAY IN PLACENTAL ARTERY ENDOTHELIAL CELL CAVEOLAE

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Vascular endothelial growth factor (VEGF) stimulates angiogenesis and vasodilation responsible for the dramatic rises in materno-feto interface blood flows directly linked to fetal growth and survival during pregnancy. Previous studies have shown that activation of the extracellular signal-regulated kinase (ERK2/1) pathway mediates, at least in part, the VEGF-induced angiogenic and vasodilatory responses in placental endothelial cells. It is, however, unclear how this VEGF-induced signaling pathway is organized in placental endothelial cells.

Objectives: In the present study, we used a transformed ovine fetoplacental artery endothelial cell line (SV40-OF) to test a hypothesis that the VEGF-activated ERK2/1 signaling pathway is compartmentalized in the specialized membranous inviginations termed as caveolae and disruption of caveolae interferes the VEGF-induced ERK2/1 activation in placental endothelial cells. **Methods:** SV40-OF cells (passage 19–30) were cultured in MCDB-131 medium containing 10% FBS and antibiotics. Following overnight serum starvation, subconfluent (~80%) cells were treated with or without increasing concentrations of recombinant human VEGF for various times. Disruption of caveolae was achieved by pretreatment with the cholesterol depletion reagent β-cyclodextrin (β-CD, 10 mM) for 60 min. Cells ($\sim 30 \times 10^6$) were lysed for fractionation of caveolae membranes by discontinuous sucrose (45%/35%/5%) gradient ultracentrifugation using a detergent free buffer system. Activation of the ERK2/1 signaling pathways were analyzed in the caveolae membranes and total cell extracts by Western blotting with specific antibodies. **Results:** When whole cell extracts were analyzed, VEGF stimulates ERK2/1 phosphorylation in a time and dose-dependent manner. Phosphorylation of ERK2/1 maximized with treatment with VEGF (10 ng/ml) at 5–10 min, which was abrogated by pretreatment with β-CD to disrupt caveolae structure. All the molecules for compromising the ERK2/1 signaling module, Src, Ras, Raf-1 and ERK2/1, were detectable in purified caveolae membranes positive for various markers including caveolin-1, eNOS, and flotillin-1, and β-adaptin. In the caveolae, treatment with VEGF (10ng/ml) dramatically increased the amounts of phosphorylated ERK2/1 without altering total ERK2/1 in a time-dependent manner similar to that in the total cell extracts, which also maximized at 5–10 min. In cells pretreated with β-CD, VEGF failed to stimulate ERK2/1 phosphorylation and also did not alter total ERK2/1 in the caveolae. **Conclusions:** Our data demonstrate that VEGF activates the ERK2/1 signaling pathway in the caveolae and the integrity of caveolae structure is essential for VEGF activation of the ERK2/1 signaling pathway. Thus, we conclude that caveolae serves as a platform for compartmentalizing the VEGF-induced ERK2/1 signaling pathway in placental endothelial cells (Supported by NIH RO1 grants HL74947 and HL70562).

RELATIONSHIP BETWEEN MATERNAL DIETARY INTAKE, HORMONE LEVELS AND PLACENTAL TRANSPORT FUNCTIONS IN OBESE WOMEN

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Objectives: A high pre-pregnancy BMI increases the risk of fetal overgrowth, which is associated with traumatic birth injuries and a susceptibility to develop obesity, diabetes and hypertension later in life. The mechanisms underlying fetal overgrowth in mothers who are overweight or obese are not well established. We hypothesized that placental nutrient transporters are up-regulated in pregnancies of overweight and obese women and that these changes are related to maternal dietary intake and serum concentrations of metabolic hormones in both 1st and 3rd trimester. **Methods:** Pregnant women with BMI ranging from 17–44 were recruited in 1st trimester. Maternal fasting blood samples and dietary interviews were obtained in 1st and 3rd trimester and, in a subset of subjects, placentas were collected at term deliveries. Syncytiotrophoblast microvillous plasma membranes (MVM) were isolated and the activity of the amino acid transporter systems A and L as well as the protein expression of the system A isoform SNAT2 and glucose transporter 1 (GLUT1) were examined. **Results:** In both 1st and 3rd trimester maternal dietary intake of total energy, protein and total fat were positively correlated with early pregnancy BMI ($n = 49$, $p < 0.05$). Similarly, maternal leptin and insulin levels in both 1st and 3rd trimester were positively correlated to maternal BMI. Maternal 1st and 3rd trimester insulin levels and 3rd trimester serum IGF-I were positively correlated to birth weight ($n = 16–19$, $p < 0.05$), whereas maternal 3rd trimester IGFBP-1 and adiponectin were inversely correlated to birth weight ($n = 17–19$, $p < 0.05$). Protein expression of MVM SNAT2 was significantly and positively correlated ($n = 18$, $p < 0.05$) to birth weight, as well as to 1st and 3rd trimester maternal insulin and 3rd trimester maternal IGF-I levels, and inversely correlated to both 1st and 3rd trimester maternal adiponectin. In contrast to SNAT2, MVM GLUT1 protein expression was not associated to any of the measured parameters. We found a strong and inverse correlation between protein intake in 3rd trimester and MVM system A activity, but not MVM SNAT2 expression. Leucine uptake into MVM was positively correlated ($n = 14$, $p < 0.05$) with 1st trimester adiponectin and negatively correlated with 3rd trimester resistin. **Conclusion:** A high early pregnancy BMI was associated with increased birth weight and placental protein expression of the System A isoform SNAT2, which could contribute to fetal overgrowth. Increased maternal levels of insulin and IGF-I, which have been shown to stimulate system A in vitro, may represent a key link between maternal adiposity and increased placental SNAT2 expression. Obesity had a larger influence on MVM SNAT2 expression than on system A activity, suggesting that SNAT isoforms are differentially regulated by maternal dietary intake and hormones.

PEROXYNITRITE INDUCES RELAXATION OF CHORIONIC PLATE ARTERIES

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Background: Pre-eclampsia (PE) and fetal growth restriction (FGR) are associated with fetoplacental vascular dysfunction and oxidative stress. Peroxynitrite, a toxic oxidant species resulting from the interaction between superoxide and nitric oxide, is implicated in vascular dysfunction in many diseases. Nitrotyrosine, a marker of peroxynitrite formation is increased in the placental villous vasculature in PE[1] and diabetes[2] consistent with chronically elevated peroxynitrite. **Objectives:** To test the hypothesis that peroxynitrite (a) modulates vascular reactivity and (b) generates nitrotyrosine in chorionic plate vessels of normal pregnancy. **Methods:** Term placentas were collected from uncomplicated pregnancies following vaginal delivery or Caesarean section. Chorionic plate arteries were dissected mounted on a wire myograph, normalized at $0.9L_{5.1kPa}$ (~20 mm Hg) and equilibrated (37°C; 20 minutes in $5\%O_2/5\%CO_2$ balance N_2). To examine the effect on basal tension arteries were treated with authentic peroxynitrite (10^{-4} M, $n = 6$ vessels, $N = 3$ placentas) or the control diluent for 30 minutes. To examine a possible relaxatory effect, paired arteries were constricted with a sub-maximal concentration of U46619 (thromboxane mimetic; EC_{80}) and then exposed to peroxynitrite (10^{-4} M; $n = 12$ vessels, $N = 6$ placentas) or the control diluent ($n = 12$ vessels, $N = 6$ placentas). Localization of nitrotyrosine residues in chorionic plate vessels was examined by immunohistochemistry. Chorionic plate cryosections ($N = 5$ placentas) were treated with authentic peroxynitrite or the control diluent for fifteen minutes prior to immunostaining for nitrotyrosine, using standard immunohistochemical techniques. Intensity of staining was semi-quantitatively analysed by three independent observers using blinded scoring. **Results:** Peroxynitrite had no effect on the basal tension of chorionic plate arteries, but induced a reversible relaxation of pre-constricted arteries compared to diluent controls (treatment v. controls; median (range), maximal relaxation to 26 (21–73) % vs. 104 (93–125) % of U46619 EC_{80}, $P < 0.05$ Mann–Whitney U test). Chorionic plate sections treated with peroxynitrite exhibited intense discrete staining of the vascular endothelium which was absent in diluent treated sections ($P < 0.05$ Wilcoxon Signed Rank test). **Conclusion:** Peroxynitrite induced relaxation of pre-constricted chorionic plate arteries and protein tyrosine nitration in the endothelium of chorionic plate vessels. The localization of nitrotyrosine residues in the vascular endothelium suggests that the altered vascular reactivity following peroxynitrite exposure is via an effect on endothelial cell function. Overall, our results are consistent with the observation of increased nitrotyrosine in the villous vasculature in PE [1] and a role for protein nitration in the altered regulation of vascular tone and blood flow in PE and FGR.

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PREECLAMPTIC PREGNANCIES AT HIGH ALTITUDE HAVE BETTER SPECIFIC MORPHOMETRIC DIFFUSION CAPACITY THAN HEALTHY PREGNANCIES AT LOW AND HIGH ALTITUDE

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The morphology of placentas from healthy pregnancies differs between residents of high and low altitude.

Our Objectives: To determine how the morphology of preeclamptic (PE) high altitude (HA, 3100 m) placentas differed from that of healthy placentas from low (LA) and HA pregnancies. **Methods:** Placentas were collected at term from healthy (HHA and PE (PE HA) pregnancies at HA (3,100 m) and healthy LA (HLA) pregnancies. Formalin-fixed paraffin-embedded sections (3–4 μm) and gluteraldehyde-fixed resin-embedded sections (1 μm) were analyzed. **Results:** Placentas were smaller in the PE HA (490 g) and HHA (517 g) groups vs. HLA (734 g). The percent of gas exchange volume was equivalent between all placentas, however the absolute volume of gas exchange was lower in PE HA (167 cm^3) and HHA (198 cm^3) vs. LA placentas (297 cm^3), as were stem villous volumes. Within the parenchyma, cytotrophoblast, capillary and stroma volumes were lower in PE HA and HHA vs. HLA. Villous and capillary surface areas were lower in PE HA (9.8 m^2, 142 m^2) and HHA (10.1 m^2, 169 m^2) vs. LA (17 m^2, 300 m^2), but the ratio was equivalent between groups. Capillary length was lower in PE HA (464 km) and HHA (534 km) vs. HLA (972 km). Morphometric diffusion capacity of oxygen was greater in PE HA (6.1×10^{-10} cm^2/sec/mbar) and HHA (6.1×10^{-10} cm2/sec/mbar) vs. LA (5.1×10^{-10} cm^2/sec/mbar). Birth weight in PE HA (2.64 kg) was lower than HLA (3.88 kg), while HHA (3.22 kg) was equivalent between the two conditions. Specific morphometric diffusion capacity greatest in PE HA (2.4×10^{-10} cm^2/sec/mbar/kg) and HHA (1.9×10^{-10} cm^2/sec/mbar/kg) was greater than HLA (1.4×10^{-10} cm^2/sec/mbar/kg). **Conclusion:** These data suggest similar placental morphometery in healthy and preeclamptic pregnancies at high altitude, with less paryncyhma than low altitude. However, morphometric diffusion capacity is better than at low altitude and specific morphometric diffusion capacity is greatest in preeclamptic high altitude pregnancies.

TEA-SENSITIVE K^+ CHANNELS REGULATE hCG SECRETION AND PRODUCTION BY HUMAN VILLOUS CYTOTROPHOBLAST CELLS *IN VITRO*

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Objectives: Maintenance of human placental syncytiotrophoblast is essential for normal pregnancy and involves co-ordinated morphological and biochemical events. Differentiated syncytiotrophoblast secretes human chorionic gonadotropin (hCG); *in vitro* this acts in a paracrine manner to induce cytotrophoblast cell differentiation. K^+ channels regulate cell turnover in many tissues but their role in syncytiotrophoblast maintenance has not been evaluated. Here we test the hypothesis that TEA (tetraethylammonium)-sensitive K^+ channels participate in biochemical and morphological differentiation of cytotrophoblast cells *in vitro*. **Methods:** Placentas were obtained at term following normal pregnancy. Cytotrophoblast cells were isolated using Percoll centrifugation and maintained in culture for 66h. At 18 and 42 h, cells were treated with control medium alone or with TEA (0.1–10 mM). Medium was collected at 18 and 66 h and analysed for hCG, to assess cell secretion, and lactate dehydrogenase (LDH), to indicate cell integrity. At 66 h, cells were lysed in water and analysed for protein and hCG to estimate cell hCG production. Morphological differentiation was assessed at 66 h by immunofluorescent staining of desmosomes and nuclei. 3 independent observers quantified multinucleation as the number of nuclei in syncytial cells as a percentage of the total nuclei in a given field. The effects of TEA are expressed as percentage of control (median +/− 25 and 75 percentile; n = 5 placentas) and compared to 100% (control) using ANOVA and Wilcoxon signed rank tests. **Results:** Over 18–66h in culture, mononucleate cytotrophoblast cells fused to form multinucleated syncytia, accompanied by a 50-fold rise in hCG secretion (2 to 101mIU/ml/h/mg protein; $p < 0.02$; paired 't' test). ≤1 mM TEA had no effect on LDH release or hCG secretion or production. 10 mM TEA almost completely blocked hCG secretion (4% +2/−19 of control: $p < 0.04$) and increased LDH release (188% +168/−308: $p < 0.04$), indicating loss of cell integrity. However, 5 mM TEA reduced hCG secretion to 25% +11/−51 of control ($p < 0.04$) without affecting LDH release. This fall in hCG secretion might arise from a fall in cell production as 5 mM TEA reduced hCG in the cell lysate to 5% +0.3/−58 of control ($p < 0.03$). TEA did not alter multinucleation at any concentration used. **Conclusions:** We propose that K^+ channels blocked by 5 mM TEA (eg Ca^{2+}- activated/voltage-gated channels) regulate the production/secretion of hCG by cytotrophoblast cells. hCG secretion by trophoblast is modulated by Ca^{2+} entry through non-selective cation channels. It is possible that membrane depolarisation following K^+ channel block by TEA reduces Ca^{2+} entry and lowers intracellular Ca^{2+}, leading to altered transcription, translation or secretion of hCG. The results also suggest that neither the production/secretion of hCG, nor the activity of TEA-sensitive K^+ channels, are obligatory for cytotrophoblast cell fusion and multinucleation *in vitro.*

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