Journal of Developmental Origins of Health and Disease (2011), Volume 2, Supplement 1, S2-S34

doi:10.1017/S204017441100047X

O-1i

Pre- And Postnatal Feeding – Mechanisms in Animal Models. Sherin Devaskar. *Invited Speaker, USA.*

O-1i-1

Postnatal Growth Is Associated with DNA Methylation and Childhood Adiposity: Using Genetic Variation to Infer the Direction of Causation. Alix Groom[1], Kate Potter[1], Valerie Turcot[2], Mark S. Pearce[3], Nicholas D. Embleton[4], Caroline L. Relton[1]. *[1]Institute of Genetic Medicine, Newcastle University, United Kingdom; [2]Institute of Nutraceuticals and Functional Foods, Université Laval, Canada; [3]Institute of Health and Society, Newcastle University, United Kingdom; [4]Neonatal Service, Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom.*

The role of epigenetic mechanisms in the developmental programming of cardiometabolic disease is the focus of considerable interest. Epigenetic epidemiological studies of human populations can contribute to the evidence base in this field however reverse causation (where a disease state might perturb epigenetic patterns) might explain observed associations. Genetic variation, in the form of single nucleotide polymorphisms (SNPs), is robust to reverse causation as a disease state cannot alter genotype and can be used to infer the direction of causality.

Following gene expression analysis the *TACSDT2* gene was identified as a mediator of altered childhood adiposity in children aged 9-13 years experiencing rapid postnatal growth in the Newcastle Preterm Birth Growth Study. DNA methylation analysis of seven promoter CpG sites and genotyping of a promoter SNP (rs61779296) in the *TACSTD2* gene was undertaken in groups of 94 and 122 individuals, respectively using Pyrosequencing analysis.

Percentage methylation demonstrated associations with postnatal growth (Mann Whitney: U = -2.40, p = 0.016); *TACSTD2* gene expression (Spearman's rank correlation: rho = -0.55, p = 0.016); and childhood fat mass (Spearman's rank correlation: rho = -0.22, p = 0.037). rs61779296 genotype was strongly associated with *TACSTD2* methylation and expression levels (Kruskal Wallis: $c^2 = 9.65$, $p = 0.008$; $c^2 = 48.47$, $p < 0.001$, respectively); and childhood fat mass (Kruskal Wallis: $c^2 = 8.48$, $p = 0.014$); but not postnatal growth ($c^2 = 0.67$, $p = 0.723$). Similar findings were demonstrated between genetic markers and percentage fat mass.

The association of methylation and adiposity at age 9-13 years could plausibly arise through reverse causation. However, the clear association of *TACSTD2* genotype (in strong linkage disequilibrium with the promoter methylation) with adiposity suggests that this is not the case. Lack of correlation between postnatal growth and genotype indicate that these two factors act independently to modulate *TACSTD2* methylation, expression and subsequent adiposity.

O-1i-2

Exclusive Breast Feeding in Infancy Has a Protective Effect on Development of Knee Osteophytes: The Newcastle Thousand Families Study. Ajay Abraham[1], Kay D. Mann[1], Mark S. Pearce[1], Roger M. Francis[3], Fraser Birrell[2]. *[1]Institute of Health & Society, Newcastle University, United Kingdom; [2]Musculoskeletal Research Group, Institute of Cellular Medicine, Newcastle University, United Kingdom; [3]Institute for Ageing & Health, Newcastle University, United Kingdom.*

There has been very little lifecourse research looking at the risk of osteoarthritis (OA). Several studies have demonstrated the association between adult risk factors such as obesity and higher bone mineral density with subsequent knee OA. We performed a lifecourse analysis of risk factors for knee OA (defined by osteophytes on ultrasound) acting at different stages of life, including early life factors, among members of the Newcastle Thousand Families birth cohort.

Potential risk factors for knee osteoarthritis (including birth weight, breast feeding data and socioeconomic status) have been collected prospectively in this birth cohort of subjects aged 63 (born in May-June 1947) and an a priori conceptual framework was developed. Subjects had both knees scanned by a trained musculoskeletal sonographer. Ultrasound protocols were derived from European League Against Rheumatism (EULAR) guidelines. The presence of knee osteophytes was assessed at the tibial and femoral sites, medially and laterally. These data were analysed in relation to a range of factors from across the lifecourse using logistic regression models.

Among the 311 participants, the prevalence of knee osteophytes was 22%, 25% and 30% for right, left and "any" knee, respectively. While birth weight, exclusive breast feeding and social class at birth showed significant univariate associations with knee osteophytes, only exclusive breast feeding (among factors acting in early life) showed a significant association in the adjusted model (OR 0.81 per month; CI 0.68, 0.97; p=0.02). BMI (OR 1.11; CI 1.02, 1.20; p=0.01) and total hip bone mineral density at age 50 (OR 1.37 per 0.1 g/cm^2; CI 1.06, 1.78; p=0.02) were the factors acting in adulthood that increased the risk of knee osteophytes at age 63.

This is the first study to perform a lifecourse analysis of knee OA risk using prospectively collected data. While exclusive breast feeding is known to decrease risk of adult obesity and therefore of knee OA, this study suggests that exclusive breast feeding is an independent predictor of knee OA. The mechanism might be reduced burden of infection and inflammation through the lifecourse, a testable hypothesis.

O-1i-3

Age-Specific Effects of Faster Linear Growth and Faster Relative Weight Gain: A Comparison across Multiple Cardiometabolic Disease Risk Factors. Nanette R. Lee[1], Isabelita Bas[1], Linda S. Adair[2]. *[1]USC Office of Population Studies Foundation, University of San Carlos, Cebu, Philippines; [2]Department of Nutrition, University of North Carolina at Chapel Hill, NC, USA.*

There is concern that faster infant weight gain increases later risk of cardiometabolic diseases. However, the importance of linear growth in comparison to weight gain at different ages has not been well studied. We aimed to determine how faster length and weight gain from birth to age 21 y relate to young adult body composition, blood pressure, fasting glucose and insulin, and lipid profiles (high and low density lipoprotein cholesterol and triglycerides).

We used data from the Cebu Longitudinal Health and Nutrition Survey, a community-based longitudinal study in the Philippines. Conditional weight and length measures (residuals derived from regressing size measures at each age on all prior measures) were used to represent faster linear growth and faster relative weight gain (greater than expected weight in relation to linear growth) from 0-6, 6-12, 12-24 months, then from 2 to 8, 8-11, 11-15 and 15-21 y. Young adult risk factors were regressed on the conditional growth measures, adjusting for adult age in sex-stratified models and effect sizes for each interval were compared across outcomes (n=1432).

Chronic disease risks related to faster relative weight gain in infancy were quite small relative to effects of faster gain from later childhood to adulthood. For example, faster linear growth and weight gain prior to adolescence were not significantly related to adult HDL, LDL or triglycerides levels. Faster relative weight gain, but not linear growth after age 15 was associated with higher blood pressure, higher levels of LDL and triglycerides and increased insulin resistance. These increased risks reflect body composition, since faster adolescent weight gain strongly associates with higher adult fat mass. Faster linear growth in early infancy significantly predicted higher young adult lean mass. Patterns were similar for males and females.

Results suggest that benefits of promoting early growth in low and middle income settings outweigh the modest elevation in chronic disease risk. Further, opportunities for intervention to prevent adult chronic disease risk include the late-childhood to adolescent period.

O-1i-4

DNA Methylation of Promoter Regions of Insulin like Growth Factor 2 Is Associated with Childhood Head Circumference. Rae-Chi Huang[1], Lawrence J. Beilin[1], Anke Van Eckelen[2], Craig Pennell[1], Jeff Craig[3]. *[1]University of Western Australia, WA, Australia; [2]Telethon Institute for Child Health Research, WA, Australia; [3]Murdoch Children's Research Institute, Vic, Australia.*

Some evidence suggests that decreased adiposity at birth directs nutrition towards head growth. We hypothesize that the mechanism (at least in part) is due to altered methylation of insulin like growth factors.Our aim is to investigate the relationship between DNA methyation of Insulin like Growth factor 1 and 2 (IGF1 and IGF2/H19) promoter regions with anthropometry from birth to age 17 years.

Two hundred and ninety six 17 year olds had anthropometry, visceral and subcutaneous fat thickness and DNA methylation profiling performed for IGF1 and IGF2/H19 genes using using MassARRAY Epityping (SEQUENOM Inc., Herston, QLD, Australia) on bisulfite converted DNA. These young adults have had prospectively measured anthropometry and skin fold thicknesses at birth, one, two, three, five, eight 10, 14 and 17 years of age. Using bivariate correlation we investigated the relationship between these anthropometric measurements and DNA methylation percentage in IGF1 and IGF2 promoter regions.

Birthweight, length or head circumference was not correlated with DNA methylation of promoter regions of IGF1 or IGF2. Head circumference at one, two, three, five, eight and 10 were negatively correlated with IGF2 H19_316 CpG 11-12, 13-14 and mean H19 methylation. Length or height was negatively correlated with H19_316 CpG5-8 at ages three, eight, 10 and 14 years. IGF2 CpG 13-14 were negatively correlated with weight, chest, mid arm, subscpular, abdominal skinfolds at year 1 as well as BMI at ages one, two and three years.

These data show that IGF2 H19 methylation at age 17 years is negatively correlated with multiple anthropometric measures over serial time points, particularly head circumference and height. DNA methylation of IGF2 may influence gene expression and early childhood growth in length and head circumference. Further investigation is required to ascertain if this altered methylation is due to fetal programming effects.

O-1i-5

Maternal High Fat Diet Primes Juvenile Offspring for Increased Hepatic Inflammation and Insulin Resistance in the Non-Human Primate at 1 yr of Age. Stephanie R. Thorn[1], Karalee Baquero[1], Diana Takahashi[2], Kevin L. Grove[2], Jacob E. Friedman[1]. *[1]Pediatrics, University of Colorado, CO, USA; [2]Oregon Health Sciences University, OR, USA.*

Previously we reported that non-human primate fetuses exposed to a maternal high fat diet (HFD) had increased plasma and liver triglycerides and hepatic gluconeogenic gene activation, suggesting profound effects on fetal metabolism (JCI, 2009). Here we extended these findings to offspring at one yr of age to determine the programming effects of maternal HFD exposure.

Offspring were exposed to maternal CON or HFD from conception to weaning and assigned to a postweaning CON or HFD, resulting in four groups: CON-CON, CON-HFD, HFD-CON, and HFD-HFD. At one yr of age, growth, body composition, and metabolic parameters were measured and liver samples were analyzed for lipids and gene expression.

Body weight tended to be increased in male offspring from maternal HFD but not in females. Fasting glucose and TG were increased only in HF-CON compared to CON-CON, indicating a persistent effect of maternal HFD. Fasting insulin was increased nearly 2-fold and glucose:insulin ratio decreased 50% in offspring on postweaning HFD. QUICKI and 1/HOMA-IR insulin sensitivity indices were reduced in CON-HFD, HFD-CON, and HFD-HFD compared to CON-CON offspring. Renal adipose tissue mass increased in offspring on postweaning HFD. Expression of inflammatory markers, TNFα and IL6, were increased 2-fold in HFD-CON and HFD-HFD livers, and the effect was more pronounced in males. In the liver triglycerides, the percent of mono-unsaturated fatty acids were increased and percent PUFA were decreased in HFD offspring, irrespective of pre-weaning diet, which parallels the composition in the post-weaning diet. Interestingly, HFD-CON livers had significantly increased 20:4,n-6 (arachidonic acid), 22:6,n-3 (docosahexaenoic acid), and 18:3,n-3 (linolenic) compared to CON-CON and HFD-HFD livers. Concentration of total n-3 fatty acids were reduced by over 70% in CON-HFD and HFD-HFD livers. Livers from HFD-HFD offspring had over a 2-fold increase in the ratio of n-6 (pro-inflammatory) to n-3 fatty acids compared to other three groups.

These data indicate programmed effects of maternal HFD exposure on hepatic fatty acid composition, inflammation, and insulin resistance in juvenile monkeys. We conclude that exposure to maternal HFD beginning in-utero contributes to the progression of early metabolic disease in the juvenile non-human primate.

O-1i-6

High Fat Diet Exposure during Early Development Decreases Islet Cell Plasticity in the Juvenile Nonhuman Primate. Sarah M. Comstock, Diana L. Takahashi, Anda Cornea, M. Susan Smith, Kevin L. Grove. *Department of Neuroscience, OHSU-ONPRC, OR, USA.*

Poor nutrition during fetal development and early infancy, including High Fat Diet (HFD) consumption, leads to an increased risk for childhood obesity, diabetes and cardiovascular disease. The purpose of this study was to investigate the impact of fetal and early postnatal exposure to HFD on the development of Non-Human Primate (NHP) pancreatic islet cells.

Adult female Japanese macaques were placed on either a CTR diet - 13% calories from fat or HFD - 35% calories from fat for 2-4 years. After birth, infant animals were maintained with their mothers on the same diet then wean onto either the CTR or HFD diet for five months. Animals were necropsied at 14 months of age, yielding four postnatal groups: CTR/CTR, CTR/HFD, HFD/CTR and HFD/HFD.

Both HFD groups had a significant increase in weight gain during the post weaning period. However, body weights did not differ between juvenile offspring groups at necropsy. Both basal and glucose stimulated insulin secretion were highly elevated in HFD/HFD animals, while the CTR/HFD group displayed mild insulin resistance. Glucokinase and insulin gene expression was increased in only the HFD/HFD group. Glucose transporter 2 was elevated in the CTR/HFD juveniles. These animals also displayed increased long chain fatty acid receptor GRP40 expression. Dietary intervention during the post-weaning period served to ameliorate the effects in the HFD/CTR group.

Islet characterization demonstrated increased islet mass in both the HFD/HFD and the CTR/HFD offspring. β-cell area was elevated in both the HFD/HFD and the CTR/HFD offspring. CTR/HFD had a concomitant increase in α-cell area which served to normalize the α/β cell ratio to control levels. In contrast, the HFD/HFD group exhibited a 40% increase in the α/β cell ratio.

While both HFD groups are insulin resistant and have increased islet mass, the mechanisms that underlie these abnormalities indicate that fetal exposure to the HFD confounds pancreatic adaptations. Differences in gene expression indicate altered mechanisms of glucose processing between the two groups. Also, the difference in α/β cell ratio suggests a deregulation in paracrine function of the HFD/HFD islets, which is likely to increase susceptibility of these young animals to diabetes. These studies demonstrate that in-utero exposure to the HFD leads to decreased islet cell plasticity in response to chronic post-natal HFD consumption.

O-1ii

Gene-Environment Interactions in Placentation and Pregnancy Success. Claire Roberts. *Invited Speaker, Australia.*

O-1ii-1

Maternal High Fat Diet Decreases Placental Blood Flow and Increases the Frequency of Stillbirth in a Non-Human Primate Model of Excess Nutrition. Antonio Frias[1], Terry K. Morgan[1], Juha Rasanen[1], Anne Evans[2], Kent Thornburg[1], Kevin Grove[2]. *[1]Oregon Health & Science University, USA; [2]Oregon National Primate Research Center, USA.*

Pre-pregnancy maternal obesity confers an increased risk of stillbirth; however, the mechanisms whereby excess maternal nutrition affects the placenta are poorly understood. We have shown that pregnant female monkeys exposed to a high fat diet (HFD) results in fetal inflammation and lipotoxicity. We used a nonhuman primate (NHP) model to determine the effect of chronic high-fat diet (HFD) on uterine and placental hemodynamics and placental histology.

A total of 24 adult female Japanese macaques were separated into two groups: the control (CTR) group (n=9) was fed a standard monkey chow that provides 14% of calories from fat. The HFD group was maintained on a diet that supplies 32% calories from fat. On gestational day 120 (term 165 days), Doppler ultrasound was used to calculate uterine artery volume blood flow (cQuta), placental volume blood flow (cQuv), and umbilical artery pulsatility index (UA PI). The macaques were delivered on day 130 by cesarean section. The placenta samples were processed and stained by H&E and were graded by a placenta pathologist blinded to the treatment group. One-way ANOVA was used for statistical analysis.

Animals fed the HFD segregated into diet resistant (HFD-R, n=6), or diet sensitive (HFD-S, n=9) based on body weight and insulin resistance. HFD animals showed a 38-56% reduction in cQuta ($p<0.05$). HFD consumption by obese mothers with hyperinsulinemia (HFD-S) led to a 32% reduction in cQuv ($p<0.05$) and an increased incidence of placental infarctions ($p<0.05$). In the HFD-S animals there were seven stillbirths out of 20 pregnancies ($p<0.05$) compared with one stillbirth out of a total of 26 CTR pregnancies, and one stillbirth out of a total of 13 HFD-R pregnancies. The UA PI was not significantly different amongst the groups. The placentas of HFD animals, like the fetal livers, demonstrated increased inflammation and fat deposition.

A HFD, independent of obesity, decreases cQuta and increases placental inflammation and fat deposition. Maternal obesity and insulin resistance further exacerbates the placental dysfunction and results in an increased frequency of stillbirth. Our results suggest that poor nutrition during pregnancy and not just obesity is a risk factor for adverse obstetric outcomes.

O-1ii-2

Paternal History of Exposure to Prenatal Undernutrition Alters Placental Expression of Nutrient Transporters and mTor Targets in F2 Offspring: Potential Contribution to Intergenerational Transmission of Diabetes and Obesity Risk. Elvira Isganaitis[1], Elizabeth Radford[2], Aristides Lytras[1], Michael Chen[1], Joshua Schroeder[1], Aparna Sharma[1], Anne Ferguson-Smith[2], Mary-Elizabeth Patti[2]. *[1]Joslin Diabetes Center, Harvard Medical School, MA, USA; [2]Department of Physiology, Development and Neuroscience, Cambridge University, United Kingdom.*

We have previously shown that prenatal undernutrition during an F0 pregnancy results in glucose intolerance and obesity in F1 offspring mice. Intercrossing F1 prenatally undernourished males (F1-UN) with control females leads to low birth weight, glucose intolerance and obesity in F2 males, despite normal nutrition during the second generation pregnancy. We hypothesize that disruptions in placental function and fetal metabolism may contribute to disease risk in F2 mice.

We analyzed placenta and liver from E16.5 F2 fetuses from the following breedings: (1) control females with F1-UN males (CxU) and (2) control females with control males (CxC). We used Affymetrix arrays for global assessment of gene expression in placental and liver, GSEA for pathway analysis, and qRT-PCR for validation of selected genes. Plasma fatty acids were measured using a colorimetric assay, and hepatic lipid content was assessed histologically and by mass spectroscopy (Lipomics).

Pathway analysis showed that eight gene sets related to nutrient transport ranked among the 10 most significantly enriched pathways in CxC vs. CxU placenta ($p<0.001$, FDR<0.2). These patterns were validated by qRT-PCR, with marked downregulation of genes involved in nutrient transport, including glucose transporter *Slc2a3* ($p=0.015$) and transferrin receptor *Tfrc* ($p=0.003$). Interestingly, genes involved in mTor signaling, a regulator of placental nutrient transport, were significantly upregulated in CxU placenta (*Eif4e* $p=0.002$; *Eif4bp1* $p=0.004$). In parallel, we observed significantly reduced circulating plasma fatty acids (CxC: 0.18±0.01 vs. CxU: 0.14±0.01 mmol, $p=0.01$), increased hepatic lipid content, and upregulated hepatic lipid metabolism genes (e.g. *Ppara, Mcad, Ppargc1a*, and *Ppargc1b*).

Our data demonstrate that offspring metabolic risk can result from paternal *prenatal* exposure to undernutrition, even with normal diet at the time of breeding. These paternal-lineage effects are likely to be mediated by alterations in epigenetic marks in the developing paternal germ cells, which ultimately modulate placental function and fetal metabolism.

O-1ii-3

Altered Expression of L-Arginine/NO-Related Enzymes in Cultured Endothelial Cells from IUGR Placenta Is Accompanied by Specific DNA Methylation Changes. Bernardo J. Krause[1], Paula Costello[2], Emma S. Garrat[2], Karen A. Lillycrop[2], Luis Sobrevia[1], Mark A. Hanson[2], Paola Casanello[1]. *[1]PRL-CMPL, Division of Obstetrics & Gynaecology, School of Medicine, Pontificia Universidad Católica de Chile, Chile; [2]Developmental Origins of Health and Disease Division, University of Southampton, United Kingdom.*

Human umbilical vein endothelial cells (HUVEC) from Intrauterine Growth Restriction (IUGR) placentae have reduced nitric oxide (NO) synthesis, along with decreased expression of endothelial NO synthase (eNOS) and increased expression of Arginase-2 (Arg-2). In endothelial cells (EC) eNOS expression is strongly regulated by epigenetic mechanisms, however it is not known if they are involved in the altered expression of eNOS and Arg-2 in IUGR HUVEC, and whether this expression pattern occurs in other placental EC. We studied eNOS and Arg-2 expression, and the methylation pattern of their gene promoters in HUVEC and placental artery EC (PLAEC).

HUVEC and PLAEC were obtained from Normal (N) and IUGR placentae. Protein and mRNA levels were determined by Western blot and RT-PCR. Specific CpG methylation of eNOS (10 CpGs) and Arg-2 (12 CpGs) gene promoters was determined by pyrosequencing of bisulfite treated DNA. CpGs were numbered according to their position relative to the transcription start site.

Protein and mRNA levels of eNOS were lower, whilst Arg-2 levels were higher in IUGR vs N-EC. The eNOS promoter of IUGR-PLAEC showed higher methylation at CpG -5375 (43.3 ±2.3%) and CpG -95 (18.5 ±2.0%) vs N-PLAEC (32.7 ±1.8% and 9.6 ±0.1% respectively), whilst the methylation at CpG -352 was reduced in IUGR (18.5 ±3.2% vs 31.6 ±4.3%). In IUGR-HUVEC only CpG -352 showed altered methylation (14.4 ±2.6%) vs N-HUVEC (6.5 ±1.3%). Arg-2 promoter of IUGR-PLAEC showed increased methylation at CpG -471 (22.5 ±4.2%) and decreased methylation at CpG -434 (4.2 ±1.2%) vs N-PLAEC (10.6 ±1.1% and 13.7 ±1.3% respectively). Comparison of eNOS promoter methylation patterns between HUVEC and PLAEC showed that N-EC present specific sites of differential methylation which are absent in IUGR.

IUGR-EC show altered methylation patterns in both eNOS and Arg-2 promoter regions. We propose that the limited variability in DNA methylation in eNOS promoter of IUGR could suggest a reduced plasticity between arteries and veins.

Supported by FONDECYT 1080534/1110977, CONICYT ACT-73(PIA) & AT24100107, Chile. MHA supported by BHF. BK holds a CONICYT-PhD fellowship.

O-1ii-4

Autophagy and Cell Death in Placenta Associated with Maternal Obesity. Alina Maloyan, Balasubashini Muralimanohara, Steve Huffman, Leslie Myatt. *OB/GYN, UTHSCSA, TX, USA.*

Our previous studies show that maternal obesity (MO) increases inflammatory and oxidative/nitrative stress in placenta. These alterations have been shown to cause autophagy and cell death in various tissues. The aim of this study was to characterize the cellular death and survival processes occurring in placenta associated with MO.

Villous placental tissue from non-complicated pregnancies was collected after c-section (no labor) at term. Three patient groups (n=8 for each) were studied: lean (LN, BMI<25); overweight (OW, BMI=25-30), and obese (OB, BMI>30). Placentas were weighed, randomly sampled from five areas and preserved for further analysis. Autophagic structures were observed using transmission electron microscopy (TEM). The expression of autophagic (beclin1, LC3-I and II, ATG7, p62) and cell death markers (caspase-3 cleavage and TUNEL assay) was measured using Western Blot and IHC.

Using TEM, we observed increased accumulation of autophagic vacuoles (AV) in the OB group, compared to LN and OW. The morphology of AV varied from early autophagosomes with double layered membranes to multivesicular and multilamellar bodies reflecting later stages of autophagy. To reinforce these observations, we measured the expression of autophagic markers. Protein expression of Beclin1 was 2-fold higher ($p<0.05$), that of LC3-II was five fold higher ($p<0.005$) and ATG7 3-fold higher ($p<0.005$) in the OB group, compared to LN and OW. Consistent with these data, significant degradation of p62, a marker indicating an accumulation of unfolded proteins, was found in OB, compared to LN and OW tissues. Alterations in basal autophagy may lead to autophagic cell death. We found significant accumulation of cleaved caspase-3 and TUNEL-positive nuclei in the OB group compared to LN and OW. To characterize activation of autophagy at the single trophoblast cell level, monodansylcadaverine (MDC) staining of primary trophoblasts from LN and OB was performed. We found significant accumulation of punctate MDC staining in cells from OB compared to LN.

Autophagy and cell death were clearly activated in placentas from obese patients, and we hypothesize that this may represent a possible mechanism

for placental adaptation to obesity-related inflammation and oxidative/nitrative stress. Further studies are being performed to define the role of placental autophagy and cell death associated with MO. Supported by NIH HL075297; CTSA UL1RR025767.

O-1ii-5

Maternal Corticosterone (CORT) Administration Programs Placental Phenotype in Mice. Owen R. Vaughan, Amanda N. Sferruzzi-Perri, Abigail L. Fowden. *Centre for Trophoblast Research, University of Cambridge, United Kingdom.*

Prenatal overexposure to potent synthetic glucocorticoids is known to reduce birth weight and program adult physiological phenotype in rodents. This treatment also reduces placental weight in late gestation. However, little is known of the effects of natural glucocorticoids on placental phenotype, the main determinant of fetal growth. This study investigated the effect of maternal CORT treatment on the growth and amino acid transport capacity of the mouse placenta.

Female C57BL/6/J mice were given CORT (200µgml-1) in the drinking water from day (D)11-16 (MID, n=19) or D14-19 (LATE, n=10) of pregnancy (term=D21). Controls (CT, n=42) were untreated. To account for the 20% increase in ad libitum food intake seen in LATE CORT dams only, an additional nine dams were CORT treated and pair fed to control intakes from D14-19. On D16 or D19 placental amino acid transport was measured as 14C-methylaminoisobutyric acid (14C-MeAIB) clearance (Sibley *et al.* (2004) PNAS 101: 8204). After maternal euthanasia, individual fetuses and placentae were dissected and weighed. Significance ($P<0.05$) of the effect of treatment was assessed by t-test or one-way ANOVA with Bonferroni post hoc comparison as appropriate.

MID CORT treatment did not alter placental 14C-MeAIB clearance but reduced placental (CT, 99±2mg; MID CORT, 94 ± 2mg) and fetal weight (CT, 395±6mg; MID CORT 363±7mg) at D16. On D19, following three days recovery, 14C-MeAIB clearance was significantly greater than controls (CT, 115±10µlmin-1g-1; MID CORT, 159±14µlmin-1g-1); while placental weight no longer differed, fetal weight remained less (CT, 1178±13mg; MID CORT, 1116±30mg). Relative to controls, LATE CORT treatment reduced 14C-MeAIB clearance (LATE CORT, 68±10µlmin-1g-1; LATE CORT PF, 64±13µlmin-1g-1), and fetal (LATE CORT, 992±16mg; LATE CORT PF, 948±31mg) and placental weights (CT, 89±2mg; LATE CORT, 78±1mg; LATE CORT PF, 76±1mg) at D19, regardless of food intake.

This study is the first to demonstrate that the natural glucocorticoid, corticosterone, programs placental phenotype in mice in a manner dependent upon the period of overexposure. CORT administered during the period of maximal placental growth caused a 30-40% compensatory increase in 14C-MeAIB clearance after cessation of treatment whereas CORT overexposure nearer to term reduced 14C-MeAIB transport. Preventing CORT-induced maternal hyperphagia in late-pregnancy did not influence placental programming.

O-1ii-6

Restriction of Placental Vasculature in the Non-Human Primate: A Unique Model To Study Placental Plasticity and Adaptations in Fetal Hemodynamics. Victoria H.J. Roberts[1], Juha P. Rasanen[1,2], Antonio E. Frias[2], Eliot R. Spindel[3], Peta L. Grigsby[1,2]. *[1]Division of Reproductive Sciences, Oregon Health and Science University, OR, USA; [2]Department of Obstetrics and Gynecology, Oregon Health and Science University, OR, USA; [3]Division of Neurosciences, Oregon Health and Science University, OR, USA.*

The capacity of the placenta to ensure adequate fetal growth is dependent on a range of factors which include physiologic changes in utero-fetal blood flow hemodynamics. The feto-placental unit adapts to environmental influences by altering its developmental trajectory to ensure fetal survival, but these adaptations may not benefit the long term outcome of the offspring. Here we report fetal cardiovascular and regional hemodynamic alterations following placental vascular restriction in a unique non-human primate (NHP) model.

Inter-placental bridging vessel ligation (IPVL) surgery was performed in time-mated pregnant rhesus monkeys at either 80 days gestation (dGA, Early, n=4, term is 168 days) or 110dGA (Late, n=3). Control animals (n=19) were obtained from another cohort. Fetal, placental and uterine hemodynamics were serially measured using image-directed and color-pulsed Doppler ultrasound.

Despite normal umbilical venous volume (Q_{uv}) and uterine artery (Q_{UTA}) blood flows at 140dGA, IPVL at 80dGA was associated with re-distribution of fetal cardiac output, increased right to left ventricular cardiac output, and corresponding increased right ventricular fractional shortening. In addition, we observed increased shunting of blood through the ductus venosus and decreased flow, as measured by decreased pulsatility index, in the left hepatic vein which are consistent with placental compromise.

The placenta appears to have a reserve capacity which allows placental vascular resistance to remain low despite a relatively severe *in utero* insult. However, there are effects on fetal hemodynamics which will likely affect organ development and thus alter long-term outcomes. This NHP model has applications for furthering our understanding of the mechanisms of placental plasticity and regional hemodynamics, and the impact on perinatal development.

O-1iii-A

Which Fish Should I Eat? Prenatal Fish Consumption, Toxicant Exposure, and Nutrition. Emily Oken. *Invited Speaker, USA.*

O-1iii-1

Prenatal Exposures to Perfluorooctanoic Acid (PFOA) and Risk of Overweight at 20 Years of Age: A Prospective Cohort Study. Thorhallur Ingi Halldorsson[1,2], Dorte Rytter[3], Line Småstuen Haug[4], Bodil Hammer Bech[3], Inge Danielsen[1], Georg Becher[4], Tine Brink Henriksen[5], Sjurdur F. Olsen[1,6]. *[1]Center for Fetal Programming, Department of Epidemiology Research, Statens Serum Institute, Denmark; [2]Unit for Nutrition Research, Landspitali University Hospital and University of Iceland, Iceland; [3]Department of Epidemiology, School Of Public Health, Aarhus University, Denmark; [4]Division of Environmental Medicine, Norwegian Institute of Public Health, Norway; [5]Department of Paediatrics, Skejby University Hospital, Denmark; [6]Department of Nutrition, Harvard School of Public Health, USA.*

Perfluoroalkyl chemicals are persistent compounds used in various consumer products, including non-stick cookware and food packaging material. Of these compounds perfluorooctanoate (PFOA) is currently detected in humans world-wide. A recent study examining low dose developmental exposure to PFOA in mice reported higher body weight and elevated biomarkers of adiposity in female offspring at postpubertal age. The authors examined whether these findings could be replicated in humans.

A cohort of 665 pregnant women who were recruited in Aarhus Denmark in 1988 to 1989. A detailed follow-up, including clinical examination, was performed when the offspring were 20 years of age. PFOA was determined in maternal serum from week 30 of gestation.

Results: After adjustment for covariates, including maternal pre-pregnancy body mass index (BMI), smoking, education and infant birth weigh, *In utero* exposure to PFOA was positively associated with anthropometry at 20 years of age in female but not male offspring. Comparing highest to lowest quartile (median: 5.8 vs.2.3 ng/mL) in the maternal exposure distribution resulted in adjusted relative risk of 3.2 (95% confidence interval (CI): 1.4, 7.1) for overweight (BMI≥25kg/m^2) and 3.1 (95%CI: 1.4, 6.9) for having waist circumference above 88 cm among female offspring. This increase in risk corresponded to 1.6 kg/m^2 (95%CI: 0.6, 2.6) and 4.2 cm (95%CI: 1.5, 6.9) average increase in body mass index and waist circumference, respectively. Maternal PFOA concentrations were also positively associated with serum insulin and leptin and inversely associated with serum adiponectin levels in female offspring at 20 years of age.

Our findings on low dose developmental exposures to PFOA are in line with results from animal experiments suggesting potential obesogenic effects in female offspring at postpubertal age.

O-1iii-2

Postnatal Reproductive Health Following Fetal and Neonatal Exposure to the Smoking Cessation Drug Bupropion. N. E. DeLong[1], B. Poirier[1], J. R. Hyslop[1], C. J. Nicholson[1], K. M. Morrison[2], H. C. Gerstein[3], A. C. Holloway[1]. *[1]Obstetrics and Gynecology, McMaster University, ON, Canada; [2]Pediatrics, McMaster University, ON, Canada; [3]Medicine, McMaster University, ON, Canada.*

Approximately 15-20% of all women smoke while pregnant, despite intentions to quit. Smoking cessation is an important intervention for the health of the baby and the mother. Therefore smoking cessation drugs (including nicotine replacement therapy [NRT] and bupropion) are considered to be of benefit for pregnant women who are highly dependent and have been unable to quit smoking by other means. However, our lab has demonstrated that fetal and neonatal exposure to nicotine is associated with a number of adverse postnatal health outcomes, including impaired fertility in the female offspring, thus raising concerns about the safety of NRT use during pregnancy. The effect of developmental exposure to bupropion on postnatal health outcomes has not been reported. The goal of this study was to examine, in rats, the effect of fetal and neonatal exposure to bupropion on postnatal fertility of the female offspring.

Nulliparous female rats (N= 5 per group) were exposed to saline (CON) or bupropion (5mg/kg/d [BUP5] or 10mg/kg/d [BUP10]) via subcutaneous injection for two weeks prior to mating until weaning. Onset of puberty, fertility and pregnancy outcomes in the offspring were assessed at six months of age.

Fetal and neonatal exposure to the highest dose of bupropion resulted in an earlier onset of puberty in the female offspring (CON=34.8 ± 0.7d; BUP5=33.1 ± 0.4d; BUP10=32.5 ± 0.5d; p=0.01). However, the female offspring of bupropion-exposed dams did not exhibit any significant differences in time to pregnancy, gestation length, mating success, the fertility index, the live birth index, litter size, birth weight, total litter weight or survival to weaning. Interestingly, the F2 offspring of females exposed to 10mg/kg bupropion in utero and during lactation also had an earlier onset of puberty relative to control animals (CON=34.0 ± 0.3d; BUP5= 32.2 ± 0.6d; BUP10= 30.9 ±0.3d; p=0.018). Moreover, the onset of puberty in the F2 BUP10 offspring was significantly earlier than in the F1 BUP10 females (p=0.017); an effect only seen in this group.

Fetal and neonatal exposure to the smoking cessation drug bupropion, unlike NRT, does not appear to adversely affect the fertility of the female offspring. However, bupropion does appear to alter pubertal onset through an as yet unknown mechanism.

O-1iii-3

Air Pollution Exposure as a Detrimental Factor for Fetal Kidney Development. Nilsa Regina Damaceno-Rodrigues[1], Andrey Augusto Socolovithc[1], Mariana Matera Veras[2], Paulo Hilario Nascimento Saldiva[2], Elnara Marcia Negri[1], Elia Garcia Caldini[1]. *[1]Laboratory of Cell Biology, Department of Pathology, University of Sao Paulo School of Medicine, Sao Paulo, Brazil; [2]Laboratory of Experimental Air Pollution, Department of Pathology, University of Sao Paulo School of Medicine, Sao Paulo, Brazil.*

Children and fetuses are particularly vulnerable to the effects of air pollution. Although it has long been known that gestational exposure to high levels of particulate matter (PM) is associated with intrauterine growth restriction and low birth weight, none of the previous studies have evaluated whether or not the development of the kidney could be affected. To test this hypothesis, we used a murine model of "real world" exposure to air pollution.

Pregnant mice were raised and completed pregnancies in chambers located at a busy crossroads in São Paulo receiving filtered (F) or non-filtered (NF) air. The 24-hr concentration of PM inside the chambers was determined gravimetrically. At 18-days of gestation, dams were euthanized, and male fetuses (7 for each chamber) were selected (1/litter). Fetuses were weighted; the kidneys were dissected, weighted and fixed in formalin and processed for histological and stereological analysis (Cavalieri method for volumetric evaluation and optical disector for an estimation of the number of glomeruli).

The concentration of PM in F chamber was significantly lower (76%, p<0.001) than in NF chamber. Fetal weight and kidney weight were 30% smaller in NF chamber (p<0.01). The proportional decrease in both kidney and body weight indicate a symmetrical growth restriction. Both cortex and medulla of NF fetuses presented reduced volume (in mm^3); cortex: 1.450±0.247 **x** 1.026±0.360 (p<0.03); medulla: 0.758±0.116 **x** 0.494±0.152 (p<0.005). The absolute number of glomeruli in the kidney of NF fetuses (1652.04±428.4) was significantly lower (p<0.03) when compared with F group (2740,86±986.0).

These data suggest that pre-natal exposure to air pollution can be associated to an impairment of kidney development (reduced number of glomeruli) and, moreover, make evident the importance of the environment for the development of the fetus, since it is known that a low nephron number increases the risk of renal disease and hypertension later in life.

O-1iii-4

Chronic Low Dose Ethanol Exposure Alters Glucose Homeostasis. Karen M. Moritz[1], Caitlyn Heshusius[1], Kylie Quinn[1], Emelie Garbedjer[1], Karrona Tep[1], Stephen Anderson[1], Mary E. Wlodek[2], Megan E. Probyn[1]. *[1]The University of Queensland, Australia; [2]The University of Melbourne, Australia.*

Exposure to a sub-optimal uterine environment may interfere with fetal growth, physiology and metabolism and has been linked with the development of cardiovascular and metabolic diseases in adulthood. Previous studies have shown that high prenatal ethanol exposure (PEE) is associated with alterations in glucose homeostasis in adult rat offspring. However, it is unknown whether moderate PEE produces similar outcomes. We aimed to determine if moderate PEE results in impaired glucose homeostasis during adult life.

Pregnant Sprague Dawley rats were fed a liquid diet containing either 0% or 6% (v/v) ethanol. In male and female PEE rats, fasting plasma glucose and insulin concentrations were measured at two and four months of age. At four months of age, an intraperitoneal glucose tolerance test (IPGTT, 1g/kg) was performed to determine glucose tolerance and insulin secretion and an insulin challenge (1U/kg) to measure whole body insulin sensitivity.

PEE did not result in changes in offspring body weight between post-natal day one and four months of life. Fasting glucose and insulin levels were not different between groups at two months but by four months, although plasma glucose levels were similar, insulin concentrations were elevated in the PEE offspring of both sexes (P<0.05 in males and p=0.06 in females compared to control). In response to the IPGTT, the peak plasma glucose concentrations and area under the curve were similar but PEE offspring reached a higher peak plasma insulin at five mins. Responses to the ITT were similar in both groups.

PEE results in normally grown offspring which maintain normal plasma glucose concentrations up to four months of age. However, the PEE offspring require higher basal insulin for glucose homeostasis and display subtle changes in glucose handling suggesting some degree of insulin resistance at four months.

O-1iii-5

Neonatal Sertraline Exposure Impairs Cardiac Development and Increases Sympathetic Tone in Mice. Sarah Haskell, Gregory Hermann, Ran Zhang, Benjamin Reinking, Robert Roghair. *Pediatrics, University of Iowa Carver College of Medicine, IA, USA.*

While selective serotonin reuptake inhibitor (SSRI) therapy has become first line therapy for maternal depression, unknown effects on the developing fetus have limited use. Epidemiologic studies have linked third trimester SSRI use with decreased fetal growth and an increased risk of ventricular septal defects. Animal models targeting an analogous neurodevelopmental window have shown neonatal SSRI therapy decreases adult serotonin signaling and elicits features of depression. Within the central nervous system, serotonin has sympatho-inhibitory effects. We hypothesized that neonatal SSRI administration will impair cardiac development and decrease adult serotonin signaling with subsequent sympathetic activation.

C57BL/6 mice were allowed natural delivery. Pups received saline (10 ml/kg/d) or sertraline (5 or 15 mg/kg/d) on days of life 1-14. At five months, mice were implanted with a carotid or electrocardiogram radiotelemeters. Hemodynamic recordings were measured at baseline and during sympathetic blockade. Heart rate variability was assessed by Fast Fourier transformation. Indirect calorimetry was used to measure basal metabolic rates and

cardiac structure was assessed by echocardiography. Urinary excretion of 5-hydroxyindolacetic acid (5-HIAA), the main metabolite of serotonin, was quantified by ELISA.
Sertraline exposed mice were tachycardic, had exaggerated responses to sympathetic blockade, and increased low frequency heart rate variability (markers of sympathetic activation). Sertraline exposed mice had similar weights to control mice but had hyperphagia and increased basal metabolic rates. By echocardiography, sertraline exposed mice had significantly smaller left ventricular volumes in diastole and decreased stroke volumes (SSRI 34 +/- 4 microliters, control 43 +/- 6 microliters, p= 0.01). SSRI mice had decreased urinary excretion of 5-HIAA (SSRI 6.4 +/- 0.7 mcg/dl, control 9.2 +/- 1.1 mcg/dl, p=0.04).
Sertraline exposed mice have several markers of increased sympathetic activation. With echocardiographic evidence showing changes in left ventricular filling in sertraline exposed mice, further studies to delineate if the increased sympathetic activation is a result from changes in cardiac development or from a down regulation in central serotonin need to be completed.

O-1iii-B

Prenatal Obesogen Exposure and the Obesity Epidemic. Bruce Blumberg. *Invited Speaker, USA.*

O-1iv-A

Immune Programming: Possible Pathways, Existing Evidence and Implications for Global Health. Andrew Prentice. *Invited Speaker, UK.*

O-1iv-1

Early Life Exposure to a High Fat Diet Results in Histological and Molecular Changes in the Rat Prostate Which Are Transmitted Paternally to the Second Generation. Tina Bianco-Miotto[1,2], Karen Chiam[2,3], Shalini Jindal[2], Natalie Ryan[2], Siti Zulkifli[2], Simon Moretta[4], Miles De Blasio[4], Karen Kind[4], Wayne Tilley[2], Julie Owens[4]. *[1]The Robinson Institute, Research Centre for Reproductive Health, School of Paediatrics and Reproductive Health, The University of Adelaide, Australia; [2]Dame Roma Mitchell Cancer Research Laboratories, Discipline of Medicine, University of Adelaide and Hanson Institute, Australia; [3]Cancer Research Program, Garvan Institute of Medical Research, Australia; [4]Discipline of Obstetrics and Gynaecology, School of Paediatrics and Reproductive Health, The University of Adelaide, Australia.*
Excess fetal nutrition, usually due to maternal obesity, is emerging as a major risk factor for cancer. Moreover, maternal factors that increase birth weight also predict and cause adult obesity in offspring, itself associated with an increased risk of cancer. The aim of this study was to determine if early life exposure to a high fat diet (HFD) is associated with an increased risk of prostate cancer in offspring.
Female rats were fed a control (7% total fat) or HFD (23% total fat) from two weeks before mating and during pregnancy. All dams received a control diet after birth of pups. Male offspring from control dams were either fed a control diet until day 100 (control) or a HFD until day 50 (puberty) followed by a control diet until day 100 (prepubertal HFD). Male offspring from HFD dams were fed a control diet until day 100 (maternal HFD). All male offspring were sacrificed at day 100.
There was an increased incidence of prostate hyperplasia in offspring from the maternal HFD (86% vs 47% in controls; p=0.0255); and this was transmitted to the second generation through the male line (P=0.026). A maternal HFD reduced expression of genes involved in prostate cancer (*Gstp1)* and genes involved in epigenetics/imprinting (*Dnmt1*, *Igf2r*). A HFD prepuberty altered expression of genes involved in prostate cancer (*Gstp1*, *Ar*, *Cdh1*) and epigenetics/imprinting (*Dnmt1*, *Dnmt3a*, *Dnmt3b*, *Igf2r*, *Plagl1*, *Snrpn*). Prostate *Dnmt1* expression was reduced in both the first generation males and the second generation male offspring, derived from the maternal and prepubertal HFD groups.
Prostate cancer is the most common male cancer in developed countries but the major causes remain unknown. We have shown for the first time, in the rat, which spontaneously develops prostate cancer with age that an early life exposure to a HFD induces cellular, molecular and epigenetic markers of progression to cancer in the prostate of offspring, and transmission of the phenotype through the male line to the second generation.

O-1iv-2

Folic Acid Supplementation during the Juvenile-Pubertal Period in Rats Leads to Persistent Tissue-Specific Changes in the Expression and Methylation of the Tumour Suppressor Gene BRCA1. Karen A. Lillycrop[1], Lydia J. Rhodes[1], Alan A. Jackson[2], Mark A. Hanson[1], Graham C. Burdge[1]. *[1]Insitute of Developmental sciences, University of Southampton, Hampshire, United Kingdom; [2]Institute of Human Nutrition, University of Southampton, Hampshire, United Kingdom.*
Folic acid (FA) intake has been shown to influence both the epigenome during critical phases of development and the risk of developing cancer [(1)]. We investigated whether variations in FA intake in the juvenile-pubertal (JP) period induce lasting epigenetic changes in the expression of the tumour suppressor gene BRCA1, which plays a critical role in DNA repair.[(2)]
Female Wistar rats were fed a modified AIN93M semi-purified diet containing either 1 mg/kg or 5 mg/kg feed folic acid for 28 days from weaning on PN28 to PN56. All rats were then given AIN93M containing 1mg/kg folic acid. Tissues were collected on PN84. mRNA expression measured by RT PCR [(3)] , BRCA1 promoter methylation was measured by bisulphite pyrosequencing [(4)] and DNA binding by electrophoretic mobility shift assays.[(5)]
JP supplementation with FA induced tissue-specific changes in BRCA1 mRNA expression that persisted beyond the period of supplementation. BRCA1 mRNA expression was significantly increased in adipose tissue, but decreased in muscle of FA supplemented rats. Altered BRCA1 expression was accompanied by changes in the methylation of specific CpGs in its promoter. The methylation of the CpG at -15 and +14 bp relative to the transcription start site was significantly increased in adipose, whereas no changes in methylation of these CpGs was seen in muscle. Transcription factor binding to CpGs -15 and +14 was abolished by methylation.
These data show that FA supplementation during the JP period induced tissue-specific changes in the expression and methylation of BRCA1 which persist beyond the period of feeding the modified diet. Moreover, altered methylation of these specific CpGs led to altered transcription factor binding. This suggests that FA may modify cancer risk through the altered epigenetic regulation of BRCA1.
MAH receives salary support from the British Heart Foundation.
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O-1iv-B

Perinatal Factors and Risk of Male Cancers. Petra Lahmann. *Invited Speaker, Australia.*

O-1iv-3

Effect of Small Birth Size on Inflammatory Markers Associated with Cardiovascular Diseases in Young Adulthood. Gerthe F. Kerkhof, Mouna Naas, Petra E. Breukhoven, Anita C.S. Hokken-Koelega. *Pediatrics, subdivision of Endocrinology, ErasmusMC/Sophia Children's Hospital, Netherlands.*
Small birth size for gestational age (SGA) has been associated with risk factors for cardiovascular diseases (CVD) in young adulthood. It is, however, not yet known whether SGA birth affects serum levels of inflammatory markers in young adulthood, which are associated with CVD, such as C-Reactive Protein (CRP), monocyte chemotactic protein-1 (MCP-1), interleukin-8 (IL-8), and soluble vascular adhesion molecule 1 (sVCAM-1). We studied the effect of SGA birth and subsequent catch-up growth during childhood on serum inflammatory markers in young adulthood. We hypothesized that catch-up growth, rather than SGA birth, is associated with

increased serum levels of inflammatory markers in young adulthood.
In 474 adults of the PROGRAM/PREMS study aged 18-24 yr, the influence of birth weight SDS, birth length SDS and adult height SDS, was studied on serum levels of CRP, MCP-1, IL-8, and sVCAM-1, using multiple regression (MR) modeling. Models were stepwise adjusted for confounders such as gender, age, smoking, oral contraceptive use, HDLc, and LDLc. Inflammatory markers were also analyzed in subgroups: young adults born small for gestational age (birth length <-2 SDS) with short stature (adult height <-2 SDS, SGA-S) or normal stature (adult height >-1 SDS, SGA-CU), and young adults born appropriate for gestational age with normal stature (birth length and adult height >-1 SDS, controls). Subgroup analyses were adjusted for variables with a p-value <0.10 in the MR models.
In MR models, birth length SDS and birth weight SDS did not significantly influence any of the inflammatory markers. After adjustment, adult height SDS was inversely associated with CRP levels (p=0.001, R^2=0.288) and IL-8 levels (p=0.040, R^2=0.035), and positively associated with sVCAM-1 levels (p=0.005, R^2=0.363). There were no unadjusted differences in CRP, MCP-1, IL-8, and sVCAM-1 between subgroups. Also after adjustment for confounders there were no differences in CRP, MCP-1 and IL-8 between subgroups, but a tendency to lower sVCAM-1 in SGA-S compared to controls (p=0.081).
SGA birth, as well as subsequent catch-up growth during childhood, does not influence serum levels of inflammatory markers related to CVD in young adulthood. Short stature is associated with increased CRP- and IL-8 levels and decreased sVCAM-1 levels.

O-1iv-4

Macrophage Associated Cytokines Are Elevated in the Amniotic Fluid in Offspring of Fat Fed Rats. Aurora J.T. Eliasia[1], Sarah L. Henry[1], Ryan J. Wood-Bradley[1], Luise A. Cullen-McEwen[1], John F. Bertram[1], Gregory A. Rice[2], James C. Armitage[1]. *[1]Anatomy and Developmental Biology, Monash University, Victoria, Australia; [2]University of Queensland's Centre for Clinical Research, Australia.*
Consumption of a high fat diet in pregnancy has deleterious effects offspring health but the underlying mechanisms are not established. A high fat diet has been shown to induce an inflammatory state due to changes in cytokine levels. We hypothesize that a maternal high fat diet elevates cytokine concentrations in the mother and fetus, stimulating fetal growth inflammation and contributing to adult disease. We aimed to determine if pro- and anti-inflammatory cytokines are over-expressed in maternal and fetal compartments of fat fed rats in late gestation.
Female Sprague-Dawley rats (n = 6) were fed a control (7% canola oil) or high fat (HF) (3% canola oil and 20% lard) diet for three weeks prior to mating and in pregnancy. At Embryonic day E17.25 and E20 maternal blood and amniotic fluid were collected and the concentration of MIP1α, MCP-1, IL-1a, IL-1b, IL-4, IL-6, IL-10, IL-12, IL-13, IL-18, TNF-α, eotaxin, GMCSF, GROKC, RANTES, Insulin and Leptin were determined by protein solution array (Bioplex™,Bio-Rad Laboratories). ANOVA was performed with maternal diet, offspring sex and gestational age as main factors and p-values adjusted for multiple comparisons (Bonferroni).
There was no evidence for up-regulation of maternal cytokines. MCP1α ($P<0.044$), and IL-4 (0.017) concentrations in maternal plasma were lower in HF fed dams compared with controls. Compared with controls, offspring of HF dams demonstrated accelerated growth between E14.25 and E20 ($P<0.05$). MIP-1α ($P<0.005$), IL-18 ($P<0.013$) TNF-α ($P<0.0001$) were all elevated in amniotic fluid of HF fed dams indicating a pro-inflammatory macrophage associated phenotype. Interestingly the anti-inflammatory IL-10 was also elevated in amniotic fluid from HF dams ($P<0.046$). Insulin concentration was elevated ($P<0.05$) but leptin was unaltered in the amniotic fluid in offspring of HF fed dams. There was no significant effect of offspring sex for any cytokine studied.
Exposure to a high fat diet during fetal life results in accelerated growth trajectory and elevated macrophage associated pro- and anti- inflammatory cytokines in late gestation. The source of these cytokines is not the maternal circulation and we are presently determining if macrophage infiltration of placenta and membranes is involved.

O-1v

Developmental Origins of Musculoskeletal Aging. Avan Aihie-Sayer. *Invited Speaker, UK.*

O-1v-1

The Influence of Early Growth on Bone Mineral Density at Age 9-14 Years in Children Born Preterm. Sunil Bhopal[1], Kay D. Mann[2], Nicholas Embleton[1,2], Murthy Korada[1], Timothy D. Cheetham[1], Mark S. Pearce[2]. *[1]Newcastle upon Tyne NHS Foundation Trust, United Kingdom; [2]Institute of Health and Society, Newcastle University, United Kingdom.*
Preterm infants are smaller than their term counterparts and have poorer bone mineralization as measured using Bone Mineral Content (BMC) and Bone Mineral Density (BMD). Poor mineralization is suggested to continue into adolescence and adult-life, resulting in lower peak bone density and greater risk of adult osteoporosis. A variety of factors influence childhood bone mineralization, including sex, exercise, diet, hormones and growth. The aim of this study was to investigate to what extent early infant growth in preterm born children was associated with bone mineralization in later childhood.
The study included 139 preterm (≤34 weeks, ≤1750g birth weight) infants, recruited from two randomized controlled trials in Newcastle Upon Tyne, UK. Anthropometric measures were taken at clinic visits throughout childhood and follow-up whole body DEXA scans (GE Lunar iDXA) were done once each at age 9-14 years. Weight gain was calculated from weight measurements, and Z-scores created accounting for current height, weight, BMI and head circumference. Associations between the dependent and explanatory variables were assessed using adjusted linear regression.
Increasing weight gain (Z score) until two years post-term was positively associated with increasing BMD adjusted for weight, height, sex, age, puberty status, total bone area, in the arms (p=0.038), trunk (p=0.032), pelvis (p= 0.017), and total (p= 0.044) and adjusted BMC in the trunk (p=0.019) and spine (p=0.049). The same associations were not found for weight gain until term, or the twelve weeks post-term. Weight gain during the first year post term was associated with increasing pelvis BMD (p=0.046) and arms BMC (p=0.040). Z-weight itself at 1- and 2-years post-term was not predictive of adjusted bone mineralization.
Associations were seen between weight gain in the first two years post-term and BMD at age 9-14 years, remaining significant after adjustment for current bone and body size, and pubertal status, all of which are correlated with bone mineralization. These findings suggest that increasing preterm infants' weight between birth and two years may be important in improving their adolescent, and subsequently their adult, bone health.

O-1v-2

Is Type of Milk Feeding in Infancy Related to Grip Strength in Older Men and Women? Findings from the Hertfordshire Cohort Study. Sian Robinson, Karen Jameson, Shirley Simonds, Holly Syddall, Elaine Dennison, Cyrus Cooper, Avan Aihie Sayer. *MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom.*
A lifecourse approach to understanding sarcopenia is increasingly recognized - as both muscle size and grip strength in adult life are associated with weight at birth. Although animal studies show that muscle growth in the neonatal period is highly sensitive to variations in feeding[1], less is known of the influence of early postnatal nutrition on muscle growth in humans, or whether it affects muscle function in later life. Our aim was to determine whether type of milk feeding in infancy was related to grip strength in older adults in the Hertfordshire Cohort Study.
From 1911 to 1948, detailed records were kept on all infants born in Hertfordshire, UK. This included the type of milk they were fed, which was summarized at the end of the first year as breastfed only, breast & bottle-fed, or bottle-fed only. In 1998, 7106 men and women who were born between 1931 and 1939 were traced. 3225 of the men and women were interviewed at home by a trained research nurse, when information was obtained on participants' medical and social history. 2997 men and women attended clinic for further investigations. Maximum grip strength was measured using a handgrip Jamar dynamometer.
60% (1793) of the men and women were breastfed in the first year, 31% (929) were breast & bottle-fed, and 9% (275) were bottle-fed. Type of milk

feeding in infancy did not differ between the men and women studied, and was not related to social class at birth. Among men, maximum grip strength was related to the type of milk feeding, such that greater exposure to breast milk in infancy was associated with greater grip strength in adult life (P=0.015). This association remained after adjustment for the effects of a range of confounding influences (birthweight, infant growth, height, age at measurement, adult diet and level of physical activity). In contrast, type of milk feeding in infancy was not related to grip strength among the women studied (P=0.658).

Little is known of the contribution of nutrition across the lifecourse to muscle strength in older age. These data suggest that in men, differences in nutritional exposure in the early postnatal period may have lifelong implications for muscle mass and strength.

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O-1v-3

Prenatal Glucocorticoids Permanently Affect Bone Growth and Structure in the Guinea Pig. Caroline E. Bertram[1], Stuart A. Lanham[1], Philip C. Calder[1], Ricardo Rueda[2], Jose M. Lopez-Pedrosa[2], Richard O.C. Oreffo[1]. *[1]Institute of Developmental Sciences, University of Southampton, United Kingdom; [2]Discovery, Abbott Nutrition R & D, Granada, Spain.*

The hypothesis that prenatal glucocorticoid [GC] exposure disrupts skeletal development and increases bone fragility was tested in guinea pigs using μCT, with the aim of establishing a robust model for bone development. The model, which was chosen primarily because of similarities with human HPA axis development, was initially developed by SG Matthews (Toronto) laboratory, and was shown to exhibit a permanently altered HPA feedback set point, leading to possible long-term exposure to altered endogenous cortisol concentrations.

Pregnant guinea pigs were injected with 1 mg/kg dexamethasone [DEX] or saline [control] on d40/41 and d50/51 [critical times of fetal brain development]. Postnatal growth rates were monitored, and μCT analysis performed on femurs and vertebrae taken from day 0-1 (newborn)[n= 4M,4F] or day 90 (young adult)[n= 4M,4F] offspring.

Male DEX group weight gain was slower than controls ($p<0.001$). At birth, MDEX offspring bone volume was lower in the femoral head and neck, higher in the vertebra and unchanged at the midshaft ($p<0.01$). Bone density was reduced in the femoral head and midshaft, yet increased in the vertebra ($p<0.01$). Aged 90 days, MDEX femoral head & vertebral trabecular thickness, bone volume and midshaft bone volume were lower ($p<0.01$). Bone density was increased at midshaft and reduced in vertebra. Female DEX group showed lower vertebral bone density aged 1 day ($p<0.01$). At 90 days, FDEX had lower midshaft bone volume ($p<0.001$) and altered vertebral bone density ($P<0.01$).

Maternal DEX administration at times of maximal fetal brain growth affects offspring bone structure from birth into adulthood, and it is likely that these differences will increase with age, aiding investigation of underlying mechanisms. To our knowledge, this is the first bone development study to use this model in which prenatal GC injections are used as a substitute for physiological disruption in utero. The effects on bone may be direct or via actions on other signalling pathways although decreased trabecular volume indicate some direct bone effect since GCs promote osteoblast apoptosis and increase osteoclast activity. The increasing incidence of osteoporosis in an ageing population indicate that the mechanisms underlying these results warrant further investigation.

O-1v-4

Intrauterine Exposure to Maternal Obesity Inhibits Fetal Skeletal Muscle Mitochondrial Biogenesis and Insulin Sensitivity in Japanese Macaques. Carrie E. McCurdy[1], Carlos Ainza[1], Diana Takahashi[2], Kevin L. Grove[2], Julie Houck[1]. *[1]Pediatrics, University of Colorado, CO, USA; [2]Oregon National Primate Research Center, Oregon Health and Sciences University, OR, USA.*

Maternal obesity is associated with an increased and earlier risk of offspring developing obesity and type-2 diabetes (DM2). In both obese adults and lean adult offspring of DM2, reduced mitochondrial density and impaired oxidative function have been linked to skeletal muscle insulin resistance. Accordingly, it has been suggested that the fetal environment can have a profound effect on susceptibility to metabolic diseases in later life, perhaps through reprogramming of skeletal muscle metabolism. Using a non-human primate model, we investigated whether intrauterine exposure to maternal obesity or chronic high fat diet decreases fetal mitochondrial function and insulin sensitivity in skeletal muscle.

Female Japanese Macaques were fed either a control (10% fat) or a high fat/calorie diet (35% fat; HFD) for 2-4 years. Skeletal muscles were collected from fetuses (F-CON or F-HFD) that were terminated early in the 3rd trimester (G130/165). Mitochondrial biogenesis, enzyme activity, and gene expression were analyzed in whole muscle homogenates. Insulin-stimulated 2-deoxyglucose uptake (2DG), was measured in isolated muscle strips from F-CON and F-HFD at sub-maximal [0.3nM] and maximal [12nM] doses.

Mitochondrial copy number and citrate synthase activity were significantly reduced in male, but not female, fetal skeletal muscle from F-HFD vs. F-CON. Additionally, key transcriptional regulators of mitochondrial biogenesis, PGC1α, PGC1β, tFAM and Mitofusin-2, were also significantly down-regulated in F-HFD. Analysis of maximal mitochondrial complex activity showed impairments only in Complex (C) I, while CII, CII-III, and CIV activities were either increased or unchanged with maternal obesity. Ex vivo insulin-stimulated 2DG uptake at either a submaximal or a maximal insulin dose was significantly reduced in both male and female gastrocnemius and soleus muscles of F-HFD animals, demonstrating impaired insulin sensitivity and responsiveness.

Our results demonstrate that the intrauterine environment has profound effects on mitochondrial function and insulin sensitivity in skeletal muscle, which may predispose the fetus to metabolic complications in later life.

O-1v-5

Neonatal Hyperoxia Causes Decreased Angiogenic Capacity and Aortic DNA Damage in Rats: Role in Premature Vascular Aging? Fanny Huyard, Catherine Yzydorczyk, Mariane Bertagnolli, Frank Cloutier, Anik Cloutier, Anne Monique Nuyt. *CHU Ste-Justine, Univ de Montreal, QC, Canada.*

Preterm infants (8% of newborns) have decreased antioxidant defenses vs. term born babies and are exposed to high levels of oxygen (O_2) both in intensive care and as compared to the intrauterine environment. We have recently shown that neonatal hyperoxic exposure leads to hypertension, endothelial dysfunction and arterial rigidity in adult rats, all characteristic of vascular aging. To elucidate the mechanisms involved in vascular changes by studying *in vivo* and *in vitro* angiogenic capacity, markers of cellular senescence (reduced proliferation rate, DNA damage and senescence proteins) following a brief neonatal hyperoxic stress.

Sprague-Dawley pups were kept at 80% O_2 or in room air from postnatal days three to 10 (P10). At P10, angiogenesis (matrigel embedded aortic rings), microvascular density (TRITC-labeled lectine, tibialis anterior muscle), cellular proliferation (bromodeoxyuridine (BrdU) incorporation detected by immunostaining (IS)), DNA damage (IS for tumour suppressor p53 binding protein1 (53BP1)) and senescence associated protein (p53) were studied. In vitro, embryonic vascular (aortic) smooth muscle cells (EVSMC) were exposed to 40% O_2 for 24h followed by up to 96h recovery in control conditions; BrdU labeling cell proliferation assay (ELISA), IS for 53BP1 and cytotoxicity assays were realized.

At P10, O_2 exposed rats showed reduced angiogenic capacity (by 40% (24-62%, n=5), $p<0.05$), decreased microvascular density (942±70 vs. 1251±93 capillaries/mm^2; $p<0.01$, n=5), a significantly reduced aortic IS for BrdU and increased p53 and 53BP1 nuclear focis (n=7). In EVSMC (*in vitro*), immediately after O_2 exposure, BrdU was significantly reduced ($3.9x10^5 \pm 0.3x10^5$ vs. $2.3x10^5 \pm 0.3x10^5$ rlu/s, $p<0.05$, n=4) vs. controls. Nuclear focis of 53BP1 remained increased after both 24 and 96h of recovery in control conditions, suggesting persistent DNA damage; O_2 exposure was not associated with cytotoxicity.

Neonatal exposure to O_2 decreases proliferation and angiogenic capacity of developing vascular cells, causes increased DNA damage and senescence associated protein expression, suggesting premature vascular senescence. These observations precede vascular dysfunction and hypertension, and may contribute to their development. Experiments are needed to confirm perpetuated premature vascular aging in susceptible individuals exposed to hyperoxic stress.

O-1v-6

Gestation Length and Fetal Growth Have Different Effects on Corticospinal Excitability and Motor Skill Development in Children. Julia B. Pitcher[1], Luke A. Schneider[1], John L. Drysdale[1], Ryan D. Higgins[1], Michael C. Ridding[1], Nicholas R. Burns[2], Ted J. Nettelbeck[2], Ross R. Haslam[3], Jeffrey S. Robinson[1]. *[1]Robinson Institute, School of Paediatrics & Reproductive Health, University of Adelaide, SA, Australia; [2]School of Psychology, University of Adelaide, SA, Australia; [3]Neonatal Medicine, Women's & Children's Hospital, SA, Australia.*

Children born < 37 weeks gestational age (GA) often exhibit motor dysfunction at school age when compared with their term-born peers suggesting preterm birth alters normal corticospinal development. We aimed to differentiate the effects of GA and sub-optimal fetal growth (i.e. low birthweight centile (BW%) on corticospinal tract and motor skills development in non-cerebral palsy children).

Transcranial magnetic stimulation and surface electromyography were used to evoke corticospinal motor potentials (MEPs) in 132 children (aged 11.9 ± 0.8 years) born 28 – 41 weeks GA. BW% (42.1 ± 0.8%) was calculated using the GROW centile calculator. MEP stimulation thresholds (MT) and stimulus-response curves were obtained for left and right motor cortex (M1) projections to a hand muscle. Motor function was assessed with the Movement Assessment Battery for Children (MABC-2) and handgrip strength.

At least one MT was obtained from all children. Low GA was linearly associated with increased MT in both hemispheres but low BW% was independently associated with increased MT only in the right M1 ($r = -0.31$, $p = 0.008$). Stimulus-response curves could be obtained from only 70 children and, apart from MT, no other significant effect of GA or BW% was evident. Regression modelling showed that MABC-2 scores were highest in children with low left M1 thresholds born at later GA ($F[2,105] = 10.49$, $P \leq 0.0001$) but were not influenced by BW%. Grip strength correlated positively with gestation in the right hand and negatively with MT in both hands, but not with MABC scores or BW%.

Even in the mildly preterm, reduced GA and BW% are associated with reduced corticospinal excitability that is still evident in late childhood. Shortened GA affects both cortices, while low BW% preferentially reduces right M1 excitability, suggesting that brain sparing in fetal growth restriction may be hemisphere-specific. Motor skill development appears influenced more by post-natal cortical development than preterm birth per se. However, our findings may underestimate the effects of GA and BW% as children with low cortical excitability could not be fully assessed with TMS.

O-1vi-1

Diet Induced Paternal Obesity Impairs the Metabolic and Reproductive Health of Two Subsequent Generations. Julie A. Owens, Tod Fullston, Megan Mitchell, Nicole Palmer, Hussan Bakos, Michelle Lane. *School of Paediatrics and Reproductive Health, University of Adelaide, SA, Australia.*

Paternal exposure to a high fat diet and resultant obesity and diabetes in rodents, impairs glucose tolerance, due to insulin deficiency, in female offspring [1], identifying a novel pathway for intergenerational amplification of metabolic disease. We have also shown that paternal diet induced male obesity, in the absence of hyperglycemia, affects sperm motility and increase sperm DNA damage, impairing embryo development and reducing pregnancy rates, in rodents, consistent with our observations in humans [2, 3]. We therefore examined the effects of this diet induced obesity in male mice, which occurs in the absence of diabetes, on health of offspring across two generations.

Male C57BL6 mice (n=16 per diet) were fed a high fat diet (HFD, 21% fat) from five weeks of age for 12 weeks, increasing body weight and adiposity, but not altering glycaemia, when compared to males fed a nutrient matched control diet (CD, 6% fat). Males were then mated to normal weight females, and metabolic and reproductive health of the offspring (F1) (n=32/sex/diet) assessed to nine months of age. FI offspring were then mated to control fed mice and health of their offspring (F2) was similarly assessed.

Paternal high fat diet exposure and obesity reduced the number of litters produced, consistent with our previous findings of male sub-fertility. 2, 3 Paternal diet induced obesity also induced insulin resistance and obesity in male and female offspring, with earlier and more marked onset in females. Furthermore, gametes from both male and female offspring of obese fathers had impaired reproductive capacity and evidence of oxidative stress. In addition, there was subsequent intergenerational transmission of impaired reproductive function through both parental lines to the second generation and of insulin resistance through the paternal line to females and through the maternal line to males.

This is the first demonstration of high fat diet induced paternal intergenerational transmission of obesity, insulin resistance and impaired reproductive function and of their further intergenerational transmission through both parental lines. Paternal obesity alone can therefore initiate transgenerational transmission and amplification of obesity and insulin resistance and infertility.

1. Ng *et al.* 2010 Nature 467: 963
2. Bakos *et al.* 2011 Fertil Steril 95: 1700
3. Mitchell M *et al.* 2011 Fertil Steril 95: 1349

O-1vi-2

Intergenerational Transmission of Fetal Growth Restriction and Pancreatic Deficits to the Next Generation. Melanie Tran[1], Linda A. Gallo[1], Karen M. Moritz[2], Mary E. Wlodek[1]. *[1]Department of Physiology, The University of Melbourne, VIC, Australia; [2]School of Biological Sciences, University of Queensland, QLD, Australia.*

Intrauterine growth restriction increases risk of adult metabolic disease with alterations in insulin sensitivity and pancreatic β-cell mass. There is evidence for transmission of growth restriction and metabolic disease to subsequent generations. The aim of this study was to determine whether there is an intergenerational transmission of growth restriction and pancreatic β-cell deficit to the second generation male and female offspring.

Uteroplacental insufficiency was induced by bilateral uterine vessel ligation (Restricted) or sham surgery (Control) on day 18 of pregnancy in WKY rats (F0). F1 Control and Restricted female offspring were mated with normal males. F2 fetal weight was measured at embryonic day 20 and fetal pancreas collected for immunohistological analysis of β-cell and islet volume density. Another cohort of F2 offspring were studied at six and 12 months and had physiological measures including an intraperitoneal glucose tolerance test (IPGTT, 1g/kg) to measure glucose tolerance and insulin secretion and an insulin challenge (1U/kg) to determine whole body insulin sensitivity. Pancreas was collected at six and 12 months for determination of β-cell mass, islet size and area.

Restricted females had smaller F2 fetuses ($P<0.05$) at embryonic day 20 compared to those from Control mothers, with reduced β-cell and islet volume density in Restricted F2 males ($P<0.05$; female analysis ongoing). Glucose tolerance, insulin secretion and sensitivity were not different between F2 Control and Restricted offspring at six or 12 months. Whole body insulin sensitivity however was reduced at 12 months compared to six months in males and females ($P<0.05$), with females more insulin sensitive than males at 12 months only ($P<0.05$). Postnatal pancreatic analysis for determination of β-cell mass is ongoing.

Uteroplacental insufficiency resulted in F2 fetal growth restriction in male and female fetuses at day 20 of pregnancy and fetal β-cell and islet deficits in males with no impairments in glucose tolerance, insulin secretion or sensitivity in adulthood. There were gender specific effects with F2 males more insulin resistant than females at 12 months, regardless of their mother's birth weight. Being born small alters fetal growth and pancreatic development of the next generation but has no effect on adult metabolic function.

O-1vi-3

Gender Specific Patterns of Associations in Preterm and Post-Term Birth across Three Generations of Swedish Males and Females. Ilona Koupil[1], Richard Silverwood[2]. *[1]CHESS, SU/KI, Stockholm, Sweden; [2]LSHTM, London, United Kingdom.*

We analysed associations in gestational duration and risk of preterm and post-term birth across generations, and effects of birthweight-for-gestational age in grandparents on length of gestation in biological grandchildren.

The Uppsala Birth Cohort Multigenerational Study includes archive data on a representative cohort of 14,192 males and females born in Uppsala, Sweden 1915-1929 and information on descendants of the cohort obtained through linkage to routine data registers. Using a path analysis, we analysed

7915 grandparents and their 26,423 grandchildren, where the grandparent, the grandchild and the intermediate biological relation were singletons. Maternal grandmothers, maternal grandfathers, paternal grandmothers and paternal grandfathers were considered separately. Models were adjusted for social variables and fitted separately for male and female grandchildren due to evidence of effect modification by sex.

Gestational duration in grandparents was positively associated with gestational duration in their grandchildren. The observed associations are equivalent to a 0.3-0.4 ($0.01 \leq p \leq 0.07$) day increase in the grandchild's gestational duration for each additional week in the maternal grandparents' gestational duration and 0.1-0.2 ($p \geq 0.2$ in all models) day increase in the grandchild's gestational duration for each additional week in the paternal grandparents' gestational duration. Birthweight-for-gestational age in maternal grandfathers was positively associated with gestational duration in their grandchildren, while birthweight-for-gestational age in paternal grandfathers was inversely related to gestational duration in their grandsons. Distinct and gender specific patterns of statistically significant associations were observed for risk of preterm and post-term birth across generations with strongest associations observed in male grandchildren and in grandfathers. Post-term birth in maternal and paternal grandfathers was associated with higher risk of post-term birth in grandsons.

Gestational duration in maternal grandparents and weight-for-gestational age in maternal grandfathers are positively associated with length of gestation in their grandchildren while higher birthweight-for-gestational age in paternal grandfathers reduces gestational duration in their grandsons. Intergenerational associations in preterm and post-term birth are highly gender specific and generally stronger in males.

O-1vi-4

Insulin Accelerates Mesoderm Induction Via Wnt Signalling in Preimplantation Rabbit Blastocysts. René Thieme[1], Sünje Fischer[1], Maria Schindler[1], Bernd Püschel[2], Bernd Fischer[1], Anne Navarrete Santos[1]. *[1]Department of Anatomy and Cell Biology, Martin Luther University Faculty of Medicine, Halle (Saale), Germany; [2]Department of Anatomy and Embryology, Georg August University Göttingen, Göttingen, Germany.*

Growth hormones, like insulin and IGFs, greatly influence early embryogenesis. They are potent regulators of embryonic cell proliferation and differentiation. We assumed that there is an interaction between the insulin and the Wnt signalling system, and that insulin deficiency would lead to a delay in growth and embryonic disc development. The Wnt system is responsible for mesoderm development and embryo axis determination in mammals.

We used an experimental type 1 diabetes mellitus (T1D) rabbit model to investigate the effects of insulin deficiency on Wnt expression during gastrulation. T1D was induced by alloxan 10 days before mating. Six day old blastocysts were staged morphologically and analyzed for Wnt and Brachyury expression and signalling pathway activation. Blastocysts were also cultured in vitro with physiological insulin (17nM) and IGF1 (1.3) concentrations for 1-12 hours.

A later start of gastrulation indicated that blastocyst development in diabetic females was delayed. Supplementing with insulin and IGF1 accelerated gastrulation and induced the expression of the mesoderm specific transcription factor Brachyury. In vivo the Wnt molecules Wnt3a and Wnt4 were induced concurrently with mesoderm development. There was no change in Wnt3a expression in blastocysts from diabetic mothers. Wnt3a and Wnt4 proteins were mainly localized in the embryoblast. In cultured blastocysts Wnt3a and Wnt4 expression was induced by insulin. The increase in Wnt3a could be inhibited by the MEK1-specific inhibitor PD98059. The cytoplasm to nucleus ratio of beta-catenin changed to favour the nucleus when supplemented by insulin.

We show a direct relationship of insulin and the Wnt signalling pathway in rabbit blastocysts. This insulin effect on Wnt expression occurs as early as induction of the mesoderm in rabbit blastocysts.

Supported by the German Research Council (DFG; NA 418/4-2) and the Wilhelm Roux Programme, Medical Faculty, MLU

O-1vi-5

Metagenomic-Based Approach to a Comprehensive Characterization of the Vaginal Microbiome Signature in Pregnancy. Kjersti Aagaard, James Versalovic, Kevin Riehle, Jun Ma, Toni-Ann Mistretta, Cristian Coarfa, Aleksandar Milosavljevic, Joseph Petrosino. *Departments of Obstetrics&Gynecology, Pathology, Molecular Microbiology and Virology, and the Bioinformatics Research Laboratory, Baylor College of Medicine, USA.*

The human microbiome is garnishing increased interest as a means by which metabolism is regulated throughout developmental life. While current major national research efforts (*i.e.,* the NIH HMP) will enable sequence-based comprehensive characterization of the adult microbiome, how and when these diverse microbiota communities take up residence in the host and how they vary in reproductive life are unexplored at a population-wide level. In other words, while we may soon know *what* constitutes the adult human microbial guild, we will not know *how* it is established. We hypothesized that microbial abundance and diversity would differ in pregnancy, and perturbations will associate with perinatal morbidities. As a critical initial step, we sought to generate comprehensive comparative metagenomic signatures.

RNA and DNA were isolated from the vagina (introitus, post fornix, midvagina) and V5V3 16S rRNA genes were NexGen sequenced (454FLX Titanium platform). Exhaustive computational analysis of 68 samples (24 subject) from healthy gravidae (18 to 40 confirmed weeks) against 337 non-pregnant controls (64 subjects) were compared. Generated sequence was quality filtered, RDP Classifier binned relative to taxonomic depth (genus), and normalized to relative within sample percentages. QIIME was utilized to produce a phylogenetic tree and operational taxonomic units (OTU) data. Inferred relationships were derived from clinical metadata and custom visualized with machine learning approaches.

1.6 gigabytes of data containing >2.7 million reads (averaging 6,711 sequences/sample of 492nt) were generated for computational analyses. A unique vaginal microbiome signature encompassing 1,137 OTUs was observed, with significant clustering by pregnancy (non-phylogenetic and phylogenetic). Genus level data projection revealed marked depreciable abundance both by advancing gestational age and cervical proximity.

We employed state-of-the-science metagenomics and computational bioinformatics in a robust and heretofore unparalleled dataset. This serves as evidence that the vaginal microbial gene catalogue uniquely differs in pregnancy, with variance of genera across subsite and gestational age.

O-1vi-6

Maternal Fructose Intake Alters Hepatic Gene Expression and Leads to Changes in Glucose and Lipid Metabolism in Fetal and Neonatal Rat Offspring. Zoe E. Clayton, Mark H. Vickers, Cassandra Yap, Deborah M. Sloboda. *Liggins Institute, University of Auckland and the NRCGD, New Zealand.*

An adverse early life nutritional environment results in an increased risk of obesity and metabolic syndrome. However, very few studies have examined fructose intake as a model, despite its relevance to modern human diets. Fructose consumption is associated with altered hepatic function and metabolic compromise. Thus, we hypothesized that maternal fructose intake during pregnancy and lactation would alter fetal and neonatal hepatic development, predisposing offspring to metabolic dysfunction.

Pregnant rats were randomized to either control (CON) or high-fructose (FR) diets. Fructose was given in solution and comprised 20% of total caloric intake. Blood and liver samples were collected at embryonic day 21 (E21); postnatal day 2 (P2) and P10. Liver triglyceride and glycogen content was measured with standard assays. Preliminary analyses of mRNA levels of key hepatic genes regulating fructose and lipid metabolism (fructokinase, and peroxisome-proliferator activated receptor α (PPARα)) were measured using qPCR.

FR mothers were hyperinsulinemic at E21 and had higher plasma fructose levels at E21 and P10. At P10, FR mothers had higher plasma non-esterified fatty acid (NEFA) and hepatic glycogen content compared to CON. At E21, female FR fetuses had higher blood glucose, plasma fructose and hepatic glycogen but reduced β-hydroxybutyrate (BHB) levels. Male FR fetuses demonstrated no phenotype. At P2, female FR neonates had higher BHB and leptin levels, but lower plasma NEFA levels. FR male neonates

had increased NEFA levels. By P10, female and male FR neonates were equally compromised; neonates had elevated plasma fructose, altered BHB and NEFA levels and increased hepatic triglyceride content compared to CON. Preliminary analyses show that at E21, hepatic fructokinase mRNA levels were decreased in FR fetuses and at P10, PPARα mRNA levels were decreased in male FR neonates compared to CON.

These data demonstrate that maternal fructose intake during pregnancy resulted in age- and sex-specific alterations in offspring glucose and lipid metabolism that appear to be associated with changes in key genes in hepatic metabolic pathways. Further studies are underway to establish long term effects of maternal fructose intake on the health of offspring and determine whether the observed sex differences elicit different risk profiles for metabolic disease into the post-weaning period.

O-1vi-7

Postnatal Leptin Administration in Naïve Rat Pups Leads to Leptin Resistance, Hypertension and Gender Specific Myocardial Dysfunction. Anne-Maj Samuelsson[1], James Clark[2], Michael J.J. Shattock[2], Joaquim Pombo[1], Clive Coen[1], Lucilla Poston[1], Paul D. Taylor[1]. *[1]Division of Women's Health, King's College London and King's Health Partners, United Kingdom; [2]Cardiovascular Division, King's College London and King's Health Partners, United Kingdom.*

We have reported hyperphagia and hypertension in the adult offspring of obese rats associated with an exaggerated post natal leptin surge (Samuelsson *et al.* 2010). To investigate whether exposure to high concentrations of leptin in neonatal life plays an etiological role in the development of the adult phenotype, leptin was administrated to control neonates to mimic the exaggerated leptin surge.

Male and female pups of naïve Sprague-Dawley rats were treated with either exogenous rat recombinant leptin (L-Tx;n= 12) (3µg/kg, ip, PeproTech, UK) or saline (S-Tx, n= 12) from postnatal day 9-14. At one and five months of age, 24 hour anorexic responses to a leptin challenge were assessed in fasted animals (10mg/kg, ip) and blood pressure was recorded by radiotelemetry (DSIPhysioTel® PA-C10). At one month of age cardiac structure and function were assessed under anaesthesia using Micro-Echocardiography (Vevo 770® v1.2, with RMV 707B scanhead Visualsonics, Canada).

At one month of age, body weight was similar in leptin-treated (L-Tx) and control (S-Tx) groups, female L-Tx inguinal fat mass was greater than S-Tx ($p<0.05$). At one and five month, male and female L-Tx were refractory to the appetite and weight reducing effects of a leptin challenge. Micro-Echocardiography imaging revealed altered cardiac structure and function in one months old L-Tx females characterized by increased ventricular internal dimension at systole (LVID [mm] L-Tx, 3.20 ± 0.11 *v* S-Tx, 2.87 ± 0.06, n=6, $p<0.05$), reduced fractional shortening (FS [%] L-Tx, 44.3 ± 1.2 *v* S-Tx, 49.9 ± 1.4, n=6, $p<0.05$) and ejection fraction (EF [%] L-Tx, 74.9 ± 1.3 *v* S-Tx, 80.4 ± 1.4, n=6, $p<0.05$). L-Tx also demonstrated increased mean arterial blood pressure at one and five months of age ($P<0.05$).

Neonatal hyperleptinaemia causes leptin resistance at one month with subsequent hyperphagia and obesity which may reflect impaired leptin–signaling in the hypothalamus. Hyperleptinaemia also programmes a sympathetically mediated hypertension and may directly or indirectly influence cardiac structure and function in females.

Funded by the British Heart Foundation, EARNEST, and Tommy's the Baby Charity Samuelsson AM, *et al.* Hypertension. 2010 Jan;55(1):76-82.

O-2i

Programming Behavior: An Overview. Judy Cameron. *Invited Speaker, USA.*

O-2i-1

Maternal High-Fat Diet Consumption Suppresses Serotonergic System Signaling in Offspring Resulting in Increased Anxiety and Anti-Social Behavior. Elinor L. Sullivan, Ashley Kostrba, Diana Takahashi, Kristine Coleman, Kevin L. Grove. *Neuroscience, Oregon National Primate Research Center, OR, USA.*

Given the current prevalence of obesity and the comorbidity of metal health disorders and obesity, it is critical to examine the consequences of maternal overnutrition and obesity on offspring behavior and the central pathways that regulate behavior. This study uses a non-human primate model of diet-induced obesity to examine the consequences of maternal obesity and high-fat diet (HFD) consumption on the central serotonin system, anxiety and social behavior of juvenile offspring.

Offspring from female Japanese macaques consuming either a low fat diet (13% of calories from fat) or a HFD (35% calories from fat) were examined. The Human Intruder test and novel object tests were used to assess anxiety responses to a social threat or novel item when offspring were 11 months of age. These tests were adapted from tests commonly used in children and reliably assess individual differences in primate stress response and anxiety. Behavior in social housing was assessed analysis of videography. In situ hybridization was used to assess the central serotonin system in the dorsal raphe and cerebrospinal fluid serotonin and plasma cortisol and adrenocorticotropic hormone (ACTH) levels were examined using radioimmunoassays.

At 11 months of age, offspring from mothers fed a HFD exhibited increased latency to explore novel objects indicating increased anxiety. Moreover, in social housing, offspring from HFD mothers spent more time alone and less time in contact with or engaged in play with peers. At 13 months of age, the serotonin system of HFD offspring was suppressed as indicated by a reduction in tryptophan hydroxylase 2 (THP2; the rate limiting enzyme in serotonin synthesis) in the dorsal raphe and a reduction in cerebrospinal fluid serotonin. Plasma ACTH and cortisol response to stress was similar in HFD and control offspring.

This study indicates that maternal HFD consumption causes suppression of the central serotonin system resulting in heightened anxiety and anti-social behavior in juvenile nonhuman primates. As the majority of pregnant women are overweight and consume a HFD, this study has important implications for the metal health status of future generations. Future studies will examine whether transitioning the obese mothers to a healthy control diet during gestation will reduce the risk of offspring developing anxiety and anti-social behavior.

O-2i-2

Gestational Protein Restriction Causes Morphometric Changes in the Bed Nucleus of Stria Terminalis (BNST) Neurons and Exacerbated Anxiety Behavior. Daniele B. Torres[3], Agnes Lopes[3], Ana J. Rodrigues[4], João J. Cerqueira[4], José M. Pêgo[4], Antonio F. Godinho[2], José A.R. Gontijo[3], Nuno Sousa[4], Patrícia A. Boer[1]. *[1]Department of Morphology, São Paulo State University, São Paulo/Botucatu, Brazil; [2]Toxicology Assistance Center, São Paulo State University, São Paulo/Botucatu, Brazil; [3]Department of Internal Medicine, State University of Campinas, São Paulo/Campinas, Brazil; [4]Life and Health Sciences Research Institute, University of Minho, Braga, Portugal.*

The BNST is located in a key position to regulate stress response and has been implicated in anxiety behavior. Evidence suggests that this brain region is highly plastic and responsive to deleterious stimuli in terms of neuronal morphology. In this work we characterized adult rats submitted to in utero protein restriction in terms of behavior and BNST morphology.

Pregnant females were divided into two groups: Normal Protein (NP 17%, n=20) and Low Protein (LP 6%, n=20) diets. Only male pups were used in this the study. Rats were weighted at birth and when adults were submitted to the following tests: Elevated-plus Maze (EPM), Hole Board (HB) and Open Field (OF). Animals were euthanized and the brains were processed for 3D reconstruction in Golgi--Cox staining slices. For each selected neuron, all branches of the dendritic tree were reconstructed at 600X magnification using a motorized microscope attached to a camera and Neurolucida software. A 3D analysis of the reconstructed neurons was performed using NeuroExplorer software. 47 neurons were reconstructed for the NP group and 45 for the LP group, (n= 4/group).

Our results show that LP offspring presented significant reduction in birth body weight (LP 5.72 ± 0.03g vs NP 5.94 ± 0.04g, $p=0.0002$). In the EPM test, the rats from of LP group spent shorter times in open arms when compared to NP animals (LP 31.62 ± 8.44, n=12 vs NP 57.68 ± 6.85, n=13, $p=0.02$). When the open arm time was divided by total time, compared to NP the rats from LP group remained longer time in open arms demonstrating an anxiety behavior (LP 0.05 ± 0.01, n=13 vs NP 0.14 ± 0.01, n=8, $p=0.001$). Morphological analysis showed significant reduction in dendritic tree length in BNST neurons of rats from LP group (LP 637.8 ± 37.30, n=45 vs NP 763.0 ± 50.02, n=47, $p=0.04$).

Our results demonstrate that gestational protein restriction can induce an anxious behavior in the progeny together with significant morphological changes in the BNST.
FAPESP and CAPES supported this work

O-2i-3

Executive Functioning in Young Adults Born with Very Low Birth Weight. Riikka Pyhälä[1], Jari Lahti[1], Kati Heinonen[1], Anu-Katriina Pesonen[1,2], Sonja Strang-Karlsson[2,3], Petteri Hovi[2,3], Anna-Liisa Järvenpää[2], Johan G. Eriksson[3,4,5,6,7], Sture Andersson[2], Eero Kajantie[2,3], Katri Räikkönen[1]. *[1]Institute of Behavioural Science, University of Helsinki, Finland; [2]Hospital for Children and Adolescents, Institute of Clinical Medicine, University of Helsinki, Finland; [3]National Institute for Health and Welfare, Helsinki, Finland; [4]Department of General Practice and Primary Health Care, Institute of Clinical Medicine, University of Helsinki, Finland; [5]Vasa Central Hospital, Vasa, Finland; [6]Department of General Practice and Primary Health Care, University of Helsinki, Finland; [7]Folkhälsan Research Centre, Helsinki, Finland.*

Children and adolescents born with very low birth weight (VLBW, < 1500 g) exhibit poorer executive functioning (EF) and cognitive performance than do their term born peers. However, studies in adults are scarce. We tested whether adults born with VLBW, either small (SGA, birth weight for gestational age < -2 SD) or appropriate (AGA) for gestational age, differ in executive functioning (EF) from adults born at term.

As a part of the Helsinki Study of Very Low Birth Weight Adults, 103 VLBW (37 SGA) and 105 control adults (mean age 25.0 y, range 21.4 to 29.7 y) without major neurosensory deficits performed a test battery measuring EF: the Trail Making Test, the Stroop test, a verbal fluency test, the Rey-Osterrieth Complex Figure Test, and the Connors' Continuous Performance Task. Wechsler Adult Intelligence Scale III produced an estimate for intelligence quotient (IQ).

The estimate of IQ was 102 (SD 15) among the VLBW and 111 (12) among the term born adults. In all tests of EF, when adjusted for sex, age, and parental education, the VLBW group performed worse in several indices. In these indices, the difference estimates ranged from 0.27 to 0.47 SD units (p-values < 0.05). The poorer performance was evident in both VLBW-AGA and VLBW-SGA groups. Further adjustments (for current head circumference, head circumference at birth, and current estimated IQ) had small if any effects on the results.

In comparison to controls born at term, adults with VLBW perform poorer in tests measuring executive functioning. Poorer performance pertains to both VLBW-AGA and VLBW-SGA participants.

O-2i-4

Fish Oil during Pregnancy and Offspring Risk of Attention Deficit⁄Hyperactivity Disorder and Depression: 18 Year Long Registry Based Follow up from a Randomized Controlled Trial. Marin Strøm, Sjurdur F. Olsen. *Centre for Fetal Programming, Dep. Epidemiology Research, Statens Serum Institut, Denmark.*

Fish oil contains the long chain n-3 fatty acid, docosahexaenoic acid (DHA), which is the most abundant fatty acid in the cerebral cortex. Previous studies have suggested benefial effects of maternal intake of DHA on fetal brain development and psychopathology in the offspring. Our aim was to examine whether increasing maternal intake of DHA in pregnancy may affect offspring risk of attention deficit⁄hyperactivity disorder (ADHD) and depression, as expressed by reduced frequency of filling of prescriptions for medication for ADHD and depression, respectively.

In 1990, a population-based sample of 533 women with normal pregnancies were randomly assigned 2:1 to receive four capsules with fish oil providing 1 g DHA/day (n = 266); four similar-looking capsules/d with olive oil (n = 136); or no oil (n = 131) (Olsen SF *et al.* Lancet 1992). Women were recruited and randomly assigned around gestation week 30 and asked to take capsules until delivery. Among 531 live-born children, 526 were still alive by December 2008, and attrition was less than 2%. For the analyses, children to mothers in the no oil group were excluded.

Data on prescriptions for stimulant and non-stimulant medication (ATC codes N06BA*) and for antidepressant medication (ATC codes N06A*) given to offspring was extracted from the Danish Register of Medicinal Product Statistics. Information on first prescription was used as a proxy for ADHD and depression, respectively, in the offspring.

During the 18 years that passed since childbirth, nine children from the fish oil and olive oil groups had been prescribed medication for ADHD; 25 had been prescribed antidepressant medication. There was no indication of an effect of fish oil supplementation against ADHD (hazard ratio (HR) 1.03, 95%CI: 0.26-4.11); whereas the hazard rate of depression was reduced by 53% (HR 0.47, 95%CI: 0.21-1.01; p = 0.055) in the fish oil compared with the olive oil group.

Under the assumption that intake of olive oil in the dose provided in the present study was inert, our results can be seen as supportive to the idea that increasing maternal intake of DHA in late pregnancy may decrease offspring risk of depression; whereas no such indication was observed for ADHD, possibly due to a low number of cases.

O-2i-5

Maternal Obesity, Gestational Diabetes and Physical Activity of the Adolescent Offspring. Marjaana Tikanmäki[1,2], Marja Vääräsmäki[1,2], Tuija Tammelin[3], Marika Sipola-Leppänen[1], Anneli Pouta[1,2], Anna-Liisa Hartikainen[2], Marjo-Riitta Järvelin[1,4,5], Eero Kajantie[1]. *[1]National Institute for Health and Welfare, Oulu and Helsinki, Finland; [2]Department of Obstetrics and Gynaecology, Oulu University Hospital, Oulu, Finland; [3]LIKES - Research Center for Sport and Health Sciences, Jyväskylä, Finland; [4]Imperial College, London, United Kingdom; [5]Institute of Health Sciences, University of Oulu, Oulu, Finland.*

Maternal gestational diabetes (GDM) and obesity are associated with higher levels of cardiometabolic risk factors in the offspring. We studied the association of maternal obesity, GDM and risk factors for GDM with self-reported physical activity (PA) of adolescent offspring.

We studied 16-year-old members of the population-based Northern Finland Birth Cohort 1986. When they were born, national guidelines prompted GDM screening by an oral glucose tolerance test (OGTT) in cases of glucosuria, prior GDM, suspected or previous fetal macrosomia, maternal prepregnancy body mass index (BMI) > 25 kg/m2 and age > 40 years. PA of the 16-year-old offspring, assessed by self-report considering light, brisk and commuting PA outside school hours, was summarized as metabolic equivalent hours (METhours) per week. Adequate data of 4191 adolescent offspring were analyzed by multiple linear regression adjusting for sex, height and BMI of the subject and parental educational level.

The 3446 subjects whose mothers had no indications to undergo OGTT served as controls. Compared with them, the offspring of mothers with risk factors prompting screening for GDM but normal OGTT result reported to undertake less PA expressed as METhours per week (mean difference -1.92, 95% CI -3.37 to -0.47, n=681); for offspring of GDM mothers the difference was similar but not statistically significant (-0.90, -4.75 to 2.96, n=85). When comparing the offspring of obese (BMI >30 kg/m2) mothers with controls, corresponding differences were -5.10, (-10.1 to -0.07) for the normal OGTT (n=50) and -5.64 (-16.61 to 5.34) for the GDM group (n=10). Respectively, comparing the offspring of normal weight (BMI < 25 kg/m2) mothers with controls were -1.97, (-3.60 to -0.34) for the normal OGTT (n=505) and -3.40, (-8.44 to 1.64) for the GDM group (n=49).

16-year-olds whose mothers had risk factors for GDM reported lower levels of physical activity than those whose mothers had no risk factors. The phenomenon was independent of maternal obesity or whether GDM was diagnosed in OGTT or not.

O-2i-6

Early Gestational Stress Is Associated with Specific Cognitive Deficits in Adolescents and Adults. Bea R.H. Van den Bergh[1,2], Maarten Mennes[3]. *[1]Department of Psychology, Tilburg University, Netherlands; [2]Department of Family, Health and Well-being, Flemish Government Brussels, Belgium; [3]Phyllis Green and Randolph Cowen Institute for Pediatric Neuroscience, NYU Langone Medical Center, USA.*

Indication that prenatal exposure to stress and maternal anxiety results in alteration of cognitive functioning exists from several human cohorts. For instance, a 56-59 year old cohort, exposed at the early stage of their gestation to a famine (winter 1944-45, the Netherlands), performed worse on a selective attention task; however but not on a memory and perceptual

motor learning task (de Rooij *et al.*, Proc Nat Ac Sci 2010). We compare these effects to those observed in a 15-20 year old cohort born in 1986-87 in Belgium.

At 12–22, 23–31 and 32–40 weeks of pregnancy, maternal anxiety was measured with the State Trait Anxiety Inventory (n=86). Offspring cognitive functioning was tested at ages 15, 17 and 20 using a battery of neuropsychological tasks (e.g., Go/Nogo and gambling tasks), events related potentials (ERPs; boys age 17) and functional magnetic resonance imaging measures (fMRI; boys age 20). Data were analysed with repeated measures ANCOVA, corrected for maternal postnatal anxiety.

A specific pattern of cognitive deficits in adolescents exposed to high maternal stress at 12-22 weeks of gestation was detected (p <. 05 to .01). In short, these adolescents exhibited deficits in the ability endogenously (i.e., autonomously, without external sources) to inhibit reactions to interfering, distracting stimuli or inhibit a learned response. They (e.g., performed significantly lower on sustained attention tasks and tasks with a high cognitive load). However, working memory was intact as well as performance in tasks that trigger response inhibition in an exogenous manner. ERP and fMRI measures confirmed the less optimal endogenous cognitive control function and fMRI results indicated that areas in prefrontal cortex, including inferior frontal junction, and middle frontal gyrus, were related to the level of prenatal maternal anxiety. This cognitive deficit may underlie behavioral self-regulation problems.

It is striking that in both cohorts early gestational stress specifically leads to selective attention deficits while other cognitive functions, such as working memory, remained intact. Reprogramming of the prefrontal and related areas, leading to a delay in maturation and perhaps to accelerated cognitive aging may be an underlying mechanism.

O-2ii

Programming of the Lung by Early Life Infection. Philip Hansbro. *Invited Speaker, Australia.*

O-2ii-1

Effect of Maternal Obesity on Fetal Baboon Cardiac miRNA Expression. Alina Maloyan[1], Jonathan Gelfond[2], Mark Nijland[1], Peter Nathanielsz[1], Leslie Myatt[1]. *[1]OB/GYN, UTHSCSA, TX, USA; [2]Department of Epidemiology & Biostatistics, UTHSCSA, TX, USA.*

More than 60% of women entering pregnancy are overweight or obese. Although maternal obesity (MO) is an independent risk factor for development of cardiovascular diseases (CVD) in the offspring, the mechanisms whereby MO affects the developing heart remain unclear. MicroRNAs (miRNAs) act as regulatory factors in heart development. We hypothesized that MO would affect the normal pattern of fetal cardiac miRNA expression.

Four months prior to pregnancy, healthy female baboons of similar age and weight were randomly assigned to the following dietary regime: 1. a control diet (C) containing 12% energy from fat; 0.29% from glucose, and 0.32% from fructose with energy content of 3.07 kcal/g) or 2. a maternal nutrient excess (MNE) diet containing 45% energy from fat; 4.62% from glucose and 5.64% from fructose and energy content of 4.03 kcal/g plus free access to a variety of high fructose sodas. MNE baboons were 14.4% heavier and had increased waist-hip circumference, LDL-cholesterol and body fat by DEXA compared to C. The dietary regime was carried through pregnancy. Pregnant baboons underwent c-section at 165 days of gestation (term 184) and fetal hearts were rapidly collected and snap frozen in liquid nitrogen. Total fetal cardiac RNA from C (n=6) and MNE (n=5) groups was isolated, and comprehensive miRNA sequencing and profiling were performed (LC Sciences, Houston, TX).

Comparison of cardiac miRNA profiles identified 70 differentially expressed miRNAs between C and MNE groups (p<0.05). Of those 50 miRNAs were upregulated and 20 downregulated with MNE. Interestingly, more prominent differences in miRNA expression were found between MNE and control male fetuses, than between MNE and control females, suggesting sexual dimorphism in the fetal response to MO. Among the differentially expressed miRNAs are members of the miR-1, 21, 30, and 133 families which have been previously linked to CVD in humans and mice.

The current study reveals significant aberrations in cardiac miRNA expression in the offspring of obese mothers at very early stages of life. The epigenetic modifications caused by adverse prenatal environment may represent one of the mechanisms underlying fetal programming of CVD. Supported by NIH P51 RR013986 and CTSA UL1RR025767.

O-2ii-2

Glucocorticoid Specific Changes in Fetal Heart Growth and Alterations in FGF-2 Following Prenatal Exposure in the Mouse. Lee O'Sullivan, James S.M. Cuffe, Sally Campbell, Karen M. Moritz. *School of Biomedical Sciences, The University of Queensland, QLD, Australia.*

The aim of this study was to investigate the effect of excess prenatal glucocorticoids (natural or synthetic) on murine fetal heart growth and the molecular pathways involved.

Female C57BL/6J were time mated over 3h. At embryonic day 12.5 (E12.5) pregnant dams were implanted with an osmotic mini-pump containing either corticosterone (CORT; 33μg/kg/h) or dexamethasone (DEX; 1μg/kg/h) for a 60h infusion, or left untreated (UNTR). Hearts were collected from fetuses at E17.5 and weighed. RNA was extracted and cDNA synthesized by reverse transcription. Genes related to growth and apoptosis were examined by real-time PCR. Data was analysed by treatment via a one-way ANOVA and are presented as (mean ± SEM) with the significance value of p<0.05.

Fetal body mass at E17.5 was lower in CORT compared to UNTR or DEX. Heart mass was decreased by CORT compared to UNTR or DEX (35.9 ± 2.6mg v 50.6 ± 2.9mg v 51.2 ± 1.6mg respectively; p<0.05) and there was a trend for CORT hearts to be smaller in proportion to body mass than UNTR or DEX hearts (56.0 ± 3.3mg v 65.8 ± 3.0mg v 63.4 ± 2.4mg respectively; P = 0.08).

Fibroblast growth factor-2 (FGF-2) expression was significantly lower in the CORT group compared to UNTR (0.64 ± 0.07 v 1.07 ± 0.13) but unchanged by DEX. Insulin-like growth factor-2, Platelet derived growth factor type c and the AT1a receptor was unaltered by either glucocorticoid treatment. AT2 recptor expression tended to be lower in the CORT group. There was no change in the apoptotic factors B-cell lymphoma-2 (Bcl-2) or the Bcl-2 associated protein x (Bax).

Glucocorticoid receptor expression levels were increased by DEX compared to UNTR (2.19 ± 0.24 v 1.25 ± 0.22) with no change in CORT.

These results suggests that fetal exposure to excess CORT results in reduced fetal heart growth. This attenuation in heart growth appears to be glucocorticoid specific and mediated through gene expression changes that are also glucocorticoid specific in nature. The reduced expression of FGF-2, which is critical for myocardial proliferation, as a result of excess CORT exposure may provide a mechanism for the reduced heart mass.

O-2ii-3

Higher Maternal Oily Fish Intake in Pregnancy Is Associated with Reduced Aortic Stiffness in 9 Year Old Children Assessed Using Magnetic Resonance Imaging. J. A. Bryant[1,2], C. R. Peebles[2], M. A. Hanson[1,3], S. R. Crozier[4], H. M. Inskip[4], S. M. Robinson[4], P. C. Calder[3], C. Cooper[4], K. M. Godfrey[1,4]. *[1]Southampton NIHR Nutrition, Diet & Lifestyle Biomedical Research Unit, United Kingdom; [2]Cardiothoracic Radiology, SUHT, United Kingdom; [3]Institute of Developmental Sciences, United Kingdom; [4]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom.*

Experimental studies in rats have shown that changes in maternal fatty acid intake in pregnancy are associated with increased arterial stiffness in the offspring,1 while a recent systematic review of human studies found that in adults omega-3 fish oils reduce arterial stiffness.2 We have examined the association between maternal oily fish intake in pregnancy and vascular structure in children aged nine years.

Pulse wave velocity (PWV) is an indirect measure of vascular stiffness and higher PWV is an established cardiovascular risk marker. Non-invasive assessment of aortic PWV was performed in 125 children aged nine years (70 male, 55 female) using magnetic resonance imaging (MRI) phase contrast acquisitions. The children studied were participants in the Southampton Women's Survey, in which the mother's diet had been assessed by validated food frequency questionnaires in early and late pregnancy, and maternal plasma phosphatidylcholine fatty acid composition at 34 weeks' gestation was measured by gas chromatography.

Higher maternal oily fish intake in pregnancy was associated with lower MRI aortic PWV in childhood (early pregnancy oily fish r=-0.19, p=0.047,

n=106, late pregnancy oily fish r=-0.25, p=0.005, n=125, taking account of child's sex). This finding was supported by a comparable association between higher maternal plasma docosapentaenoic acid (22: 5n-3) concentration and lower childhood PWV (r=-0.21, p=0.02, n=124).
Normal variations in maternal oily fish intake in pregnancy may alter vascular development in utero, changing arterial structure with long-term consequences for cardiovascular risk in later life.
This work was supported by funding from the British Heart Foundation and the National Institute for Health Research (Southampton NIHR Nutrition, Diet & Lifestyle Biomedical Research Unit).
1. Armitage JA, *et al.* Developmental programming of aortic and renal structure in offspring of rats fed fat-rich diets in pregnancy. J Physiol 2005;565:171-84.
2. Pase, *et al.* The effects of dietary and nutrient interventions on arterial stiffness: a systematic review. Am J Clin Nutr 2011;93:446-54.

O-2ii-4

Thyroid Hormone Suppresses IGF-I's Actions in the Fetal Heart. N. N. Chattergoon[1], S. Louey[1,2], S. S. Jonker[1,2], G. D. Giraud[1,2,3], K. L. Thornburg[1,2]. *[1]Heart Research Center, Oregon Health & Sci Univ, OR, USA; [2]Medicine (Cardiology), Oregon Health & Sci Univ, OR, USA; [3]Cardiology, Portland VA Med Cntr, OR, USA.*
Near-term fetal heart growth is marked by increased terminal differentiation (binucleation) and diminished cardiomyocyte (CMC) proliferation. Insulin-like growth factor (IGF-1) is a major stimulant of fetal CMC proliferation while tri-iodo-thyronine (T_3) appears to drive maturation of the fetal myocardium by suppressing proliferation and promoting binucleation of CMCs. The interaction of these two factors, with seemingly opposite effects, has not been investigated. Hypothesis: *Excess circulating T_3 in fetal sheep will suppress the effects of IGF-I and promote terminal differentiation of the near-term fetal heart.*
Two groups (n= 6/group) of fetal sheep were studied between 125-130 days gestational age (dGA, term ~145d): 1) Long R^3 IGF-I (IGF-I: 715mg/d i.v.) and 2) T_3+IGF-I (T_3:54mg/d + IGF-I: 715mg/d i.v.). At 130dGA the fetal heart was weighed and individual CMCs from left (LV) and right ventricles (RV) were enzymatically dissociated and isolated for analysis of terminal differentiation. Data are shown as mean±SEM.
After 5d, fetal aortic pressure did not change. Instead of the normal decrease in heart rate (HR) with gestational age, fetal HR increased in both groups. Day 5 HR is not different between groups however, d0 HR is significantly lower than d5 within each group (IGF-I vs T_3+IGF-I: 39±6.7 vs 25±4.7 beats/min higher by d5; $p<0.01$ for each group). T_3+IGF-I fetal body weight (BW) was 24% heavier (not significant) and heart weight (HW) was 37% greater ($p<0.05$); HW/BW were not different between groups. LR^3 IGF-I infusion decreased native circulating IGF-I in both groups (d0 vs d5: 143.5±25.4 vs 74.2±18.4ng/ml; $p<0.01$). T_3+IGF-I elevated peak circulating total T_3 levels for this gestational age compared to IGF-I alone (0.6±0.1 vs 0.1±0.1ng/ml; $p<0.01$). Binucleation was significantly greater in T_3+IGF-I compared to IGF-I hearts (LV: 60.5±5.5 vs 42.3±1.5%, RV: 56.3±4.6 vs 42.2±2.3%; $p<0.05$ for each ventricle). IGF-I did not change % binucleation.
1) LR^3 IGF-I infusion elevated fetal HR and suppressed normal IGF-I secretion. 2) T_3+IGF-I resulted in a more mature heart for gestational age versus IGF-I alone. In the presence of IGF-I, excess T_3 stimulated terminal differentiation (elevated binucleation) of fetal CMCs at this gestational age suggesting T_3 overcame IGF-I's normal effect on CMC maturation.

O-2ii-5

Premature Cardiac Dysfunction in Adult Rats after Neonatal Oxygen Exposure. Mariane Bertagnolli, Fanny Huyard, Keveen Youndje, Alexandre Barbier, Anik Cloutier, Anne Monique Nuyt. *CHU Ste-Justine, Univ de Montreal, QC, Canada.*
Long term vascular dysfunction and high blood pressure (BP) occur as consequence of neonatal transient oxygen (O_2) exposure in rats. Considering that the heart is in active development in the first days after birth in rats (such as in the last trimester in humans), we postulated that deleterious postnatal conditions may significantly impact the development of cardiac structure and function. This study aims to assess whether neonatal O_2 exposure leads to adult cardiac dysfunction at baseline and after angiotensinII (AngII) infusion along with changes in cardiac renin-angiotensin system expression.
Sprague-Dawley pups were kept in 80% O_2 (H, n=6) or in room air (Ctrl, n=6) from days 3-10 of life. At 12 wks, saline or AngII (100 ng/kg/day) was infused by osmotic minipumps for four wks. Intraarterial BP (mmHg) and in vivo left ventricle (LV) function were then assessed to obtain LV end-diastolic (LVEDP) and systolic (LVSP) pressures, maximum (+dP/dT) and minimum (-dP/dT) derivatives. Cardiac hypertrophy index (CHI) was determined by heart/body weight ratio. Cardiac AT1, AT2 and ACE protein expressions were assessed (Westerns).
At 16 wks, H rats show altered LV function with increased LVEDP (19.3±4.3 vs 13.6±2.6), decreased +dP/dT (1710±150 vs 1950±220) and –dP/dT (-1490±115 vs -1620±270), and elevated CHI (2.81±0.09 vs 2.66±0.15) vs. Ctrl (all $p<0.05$). Cardiac AT1 expression was increased in H (1.8±0.1 vs 1.5±0.07) and AT2 decreased (7.5±0.9 vs 6.0±0.6) vs. Ctrl (all $p<0.05$). ACE was not different. After AngII, both H and Ctrl rats displayed cardiac function changes, significantly more pronounced in H vs. Ctrl: increased mean BP (137±11 vs 124±8), LVEDP (24.2±7.5 vs 18.0±3.7), and decreased +dP/dT and –dP/dT in H vs Ctrl (all $p<0.05$), indicating an exacerbated cardiac dysfunction and heart failure after AngII challenge. CHI was also significantly increased in H group compared to Ctrl after AngII.
Neonatal high O_2 exposure leads to cardiac hypertrophy and LV diastolic dysfunction in adulthood, along with increased AT1 and decreased AT2 expression. In addition, cardiac dysfunction is exacerbated after AngII infusion in H group, indicating impaired cardiac adaptation to increased afterload challenge in rats exposed to hyperoxia as newborns. These results are of importance to understand the impact of neonatal hyperoxic stress in the origins of cardiac diseases particularly in the population of prematurely born infants.

O-2ii-6

Cardiovascular Risk in Adulthood after *In Utero* Transfusion for Fetal Anaemia. Alexandra H. Wallace[1], Stuart R. Dalziel[1,2], Kent L. Thornburg[3], Craig S. Broberg[3], Michael Jerosch-Herold[4], Otavio R. Coelho-Filho[4], Jane E. Harding[1]. *[1]Liggins Institute, University of Auckland, New Zealand; [2]Children's Emergency Department, Starship Children's Hospital, Auckland, New Zealand; [3]Heart Research Center, Oregon Health and Science University, OR, USA; [4]Department of Radiology, Brigham and Women's Hospital, MA, USA.*
In sheep, brief fetal anemia markedly alters coronary conductance, flow and architecture in adulthood. It is not known whether similar changes occur in humans. We compared cardiovascular function in adults who required *in utero* blood transfusion for treatment of fetal anemia with that of unaffected siblings.
Individuals who received *in utero* transfusion at National Women's Hospital, Auckland, from 1963-1992 (the oldest recipients in the world) were invited to participate, together with an unaffected sibling. Participants underwent cardiac MRI to assess left ventricular function and myocardial blood flow at rest, during cold pressor stress and during adenosine infusion. Cardiovascular risk factors including blood pressure, glucose tolerance and lipid status were also assessed. Data were analysed using multiple regression, adjusted for age, sex and affected/unaffected status nested within sibling groups.
Data are available for 26 participants (11 sibships). Affected participants were younger than unaffected (mean±SE, 22.1±1.9 vs 28.8±1.9y, p=0.02), but similar in sex distribution, weight, height and body mass index. Compared to unaffected siblings, affected participants had lower stroke volume (96.2±2.4 vs 115.4±2.5ml, p=0.04) and diastolic blood pressure (61.4±1.1 vs 71.4±1.1mmHg, p=0.03). They also had a trend towards lower end diastolic volume (162.2±5.1 vs 190.2±5.3ml, p=0.08).
These preliminary results provide the first evidence in humans that fetal anemia has functional consequences in adulthood. Lower stroke volume and diastolic pressure may result in decreased cardiovascular risk. However, in sheep adaptations to anemia that initially appear beneficial are detrimental under conditions of myocardial stress. Our findings of permanent alteration to cardiac and vascular function suggest a powerful and sustained effect of fetal anemia on the cardiovascular system. Further research will suggest whether these changes are detrimental to human health. These findings will have implications not only for adult survivors of fetal anemia but also for informing transfusion thresholds for the fetus and neonate.

O-2iii

Effects of Light and Melatonin on the Body Clock. Alfred Lewy. *Invited Speaker, USA.*

O-2iii-1

Prenatal Activation of PPARγ Stimulates NPY Expression in the Fetal Hypothalamus and Promotes Milk Intake in the Lamb. Jessica Gugescheff[1,2], Janna L. Morrison[1], Caroline I. McMillen[1], Beverly S. Muhlhausler[1,2]. *[1]Sansom Insitute for Health Research, University of South Australia, South Australia, Australia; [2]FOODplus Reserach Centre, The University of Adelaide, South Australia, Australia.*

Maternal overnutrition increases plasma glucose concentrations in the fetus and PPARγ mRNA expression in fetal adipose tissue and is associated with increased fat mass and altered appetite regulation in the first month of postnatal life. We have speculated that precocial activation of PPARγis the primary event through which prenatal overnutrition alters appetite regulation after birth. The aim of this study was to determine whether prenatal activation of PPARγ in the absence of increased fetal glucose concentrations results in altered expression of appetite regulating neuropeptides in the fetal hypothalamus and in a change in feeding behaviour after birth.

Osmotic pumps containing the PPARγ agonist, rosiglitazone (Rosi) or vehicle (15% ethanol) were inserted into sheep fetuses at 123-128 days gestation (term= 150 ± 3 d gestation). Sheep were divided into two cohorts; fetuses=11 and lambs=24. In situ hybridization was conducted on coronal sections of the fetal hypothalamus (Vehicle, n=4; Rosi, n=7) to determine expression of neuropeptide Y (NPY) and cocaine and amphetamine related transcript (CART) mRNA in the arcuate nucleus (ARC) of the hypothalamus. Lambs (Vehicle, n=13, Rosi, n=11) were weighed and sampled for the first five days and at two weeks milk intake was determined using a weigh-suckle-weigh protocol.

Fetuses that had been treated with rosiglitazone had a significantly higher expression of the appetite stimulating neuropeptide NPY mRNA (1.08 ± 0.1 vs 0.67 ± 0.07, $p<0.05$) in the ARC at 140d gestation. There was no effect of rosiglitazone on CART expression in the fetal ARC. Prenatal rosiglitazone treatment was associated with a higher milk intake (250 ± 13 ml vs 213 ± 36ml, $p<0.02$) and elevated plasma glucose concentrations over the first five days after birth ($F=6.85$, $p=<0.05$) in both males and females.

We have demonstrated that precocial PPARγ activation results in an increase in the expression of the appetite stimulatory neuropeptide, NPY in the fetal hypothalamus and an increase in postnatal milk intake suggesting that prenatal activation PPARγ is an important mechanism through which appetite could be programmed before birth.

O-2iii-2

The CNS Melanocortin System Contributes to Elevated Arterial Pressure in Juvenile Offspring of Obese Rats. Anne-Maj Samuelsson, Amandine Mullier, Keval Patel, Joaquim Pombo, Clive Coen, Lucilla Poston, Paul Taylor. *Division of Women's Health, King's College London and King's Health Partners, London, United Kingdom.*

Diet-induced obesity in the rat leads to sympathetically mediated hypertension and a heightened pressor response to leptin in juvenile offspring, prior to development of 'programmed' obesity (Samuelsson *et al.*, 2010). We hypothesize that maternal obesity influences central sympathetic efferent pathways in the offspring by persistent modulation of the developing melanocortin system. We have now directly investigated the role of CNS melanocortin receptors 3 and 4 (MC3/4R) in the hypertension observed.

Female Sprague-Dawley rats were fed either an obesogenic diet (Ob, 16% fat, 33% simple sugar) or standard chow (Con, 7% simple sugar, 3% fat) five weeks before mating and throughout pregnancy and lactation (n=8 per group). Offspring (OffOb, OffCon) were weaned onto standard chow. At 30 days of age, prior to development of increased adiposity, blood pressure was recorded by radiotelemetry. Following five days of baseline recording, rats were infused ICV with either MC3/4R antagonist (SHU-9119, 1nmol/h) or saline (Alzet osmotic pump; 0.5 µl/h) for seven days. After sacrifice, brains were sectioned to confirm correct cannula placement.

At baseline mean arterial blood pressure (MAP) was elevated in OffOb v OffCon (110.3±4.3 v 90.1±6.6 mmHg, $p<0.01$, n=8). MC3/4R antagonism lowered MAP to a greater extent in OffOb (-29±4 mmHg *versus* OffCon, -12±3 mmHg, $p<0.001$, n=8) (Figure 1) such that MAP was not different between groups (OffOb, 80.9±5.2 v OffCon, 77.8±3.2 mmHg, ns). Heart rate was lowered after treatment in both groups OffOb (-34.8±13.7 beats/min) and OffCon (-36.7±14.4 beats/min). SGU-9119 increased calorific intake (OffOb 94% and OffCon 44%, $p<0.001$) leading to greater weight gain in OffOb (OffOb 56.4±1.4 g *v* OffCon, 47.4±1.6 g, $p<0.01$, n=8). Saline treatment did not affect calorific intake, weight gain, HR or MAP. Responses in males and females were similar.

The CNS melanocortin system contributes to sympathetic efferent activity and elevation of MAP in offspring of obese dams and may play a key role in the early life origins of sympathetically mediated hypertension.

Funded by the British Heart Foundation, EARNEST, and Tommy's the Baby Charity Samuelsson AM *et al.* Evidence for sympathetic origins of hypertension in juvenile offspring of obese rats. Hypertension. 2010 Jan;55(1):76-82.

O-2iii-3

Circadian Variation in Nutrient Transporter Expression in Rat Placenta. Brendan J. Waddell, Michaela D. Wharfe, Jessica L. Lewis, Peter J. Mark. *School of Anatomy & Human Biology, The University of Western Australia, Western Australia, Australia.*

Rhythmic expression of clock genes drives circadian variation in various physiological processes both centrally and in peripheral tissues such as the liver. We recently demonstrated the rodent placenta expresses all of the canonical clock genes in a highly zone-specific pattern and with some circadian variation (albeit less than in other tissues). Because clock genes can influence expression of nutrient transporters in peripheral tissues, including hepatic Glut2, the present study examined whether expression of placental nutrient transporters varies in a circadian pattern.

Pregnant rats (n=6/time point) were sampled over days 21-22 of gestation (term = day 23). Samples of junctional (JZ) and labyrinth (LZ) zones of the placenta were collected at 0800, 1400, 2000 and 0200 h, which equate to zeitgeber times ZT1, ZT7, ZT13 and ZT19 respectively. JZ and LZ expression of genes encoding transporters for glucose (Glut1, Glut3, Glut4), amino acids (Snat1, Snat4) and fatty acids (CD36) were measured by quantitative PCR. Data were analysed by one- or two-way ANOVA, as appropriate.

Both zones of the rat placenta expressed each nutrient transporter, with zonal differences clearly evident for Glut3 and Snat4 ($P<0.001$). Glut3 was 6-fold higher in LZ than in JZ, while Snat4 was 5- to 6-fold higher in the JZ. LZ expression of Glut1, Glut4, Snat1, Snat4 and CD36 all varied with time of day (1.3- to 4-fold increases across the day; $p<0.05$). The most marked circadian variation in LZ expression occurred in Snat1, which exhibited a peak at ZT1 that was 4-fold higher than the trough at ZT19. In contrast, Snat4 expression was highest at ZT13 and lowest at ZT1. Glut1 expression peaked at ZT13 while Glut4 was highest at ZT7 and both had their lowest expression at ZT19. CD36 expression peaked at ZT7 and was lowest at ZT19. No detectable rhythms were observed within the JZ for any of the transporters.

In conclusion, these data show that the placental LZ (the site of maternal-fetal exchange) exhibits marked circadian variation in nutrient transporter expression late in gestation, although changes in the Snat transporter isoforms may counterbalance each other. It will be crucial to assess whether these circadian transcriptional changes impact on the transplacental passage of nutrients.

O-2iii-4

The Role of µ-Opioid Receptors in the Regulation of Macronutrient Preferences and Body Weight Gain. Zhi Yi Ong[1], Beverly Muhlhausler[1,2]. *[1]Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia; [2]FOODplus Research Centre, School of Agriculture, Food and Wine, University of Adelaide, Adelaide, Australia.*

Recent studies have shown that perinatal exposure to palatable foods programs the preference for high-fat foods in the offspring after birth. In adults, activation of µ-opioid receptors (Mu) stimulates palatable food intake. In this study we investigated the hypothesis that maternal 'junk-

food' feeding during pregnancy and lactation programs an increased fat preference in the offspring through altered expression of Mu within the mesolimbic reward system.

Dams were fed a cafeteria diet (Junk Food, JF, ~45%fat, n=6) or control chow (Control, 5%fat, n=6) from 4wks pre-mating to end of lactation. Offspring from Control and JF dams were given free access to both control and cafeteria diet from weaning until 3mo of age and food preferences determined. Offspring were weighed weekly. At post-mortem (6wks and 3mo of age), the brain was collected and nucleus accumbens (NAc) and ventral tegmental area (VTA) isolated. mRNA expression of Mu in the NAc and VTA was determined by qRT-PCR.

Both male and female offspring of JF dams consumed more fat than Controls from weaning until adulthood (Males: 16±0.6 g/day vs 11±0.8g/day; Females: 19±1.3g/day vs 13±0.4g/day; $p<0.01$). At 6wks of age, JF offspring were lighter than Controls independent of sex (168.2±6.6g vs 214.5±8.8g; $p<0.01$) but body weight was no longer different from Controls at 3mo. JF offspring had higher Mu mRNA expression in the NAc than Controls at 6wks of age (Control: 5.8x10-3±4.5x10-4 vs 3.5x10-3±4.4x10-4:; $p<0.01$), but was reversed at 3mo (1.3x10-2±3.7x10-3 vs 3.7x10-2±1.0x10-2,$P<0.05$). In the VTA, Mu mRNA expression tended ($P<0.07$) to be higher in JF offspring than Controls at 6wks of age. Mu mRNA expression in the NAc was positively correlated with mean fat intake ($r= 0.524$; $p<0.03$) and inversely correlated to body weight ($r= -0.565$; $p<0.02$) at 6wks of age.

This study suggests that an early increase in Mu expression, and thus increased opioid signalling within the mesolimbic reward system, may be responsible for the increased fat preference in offspring exposed to a palatable diet during the perinatal period. It is important in future studies to determine the critical developmental windows for the programming of the mesolimbic reward circuitry, and whether these effects can be reversed by interventions applied after birth.

O-2iii-5

Hypothalamic Metabolic Sensors: Mechanisms for Programmed Hyperphagia and Adult Obesity in Offspring of Maternal Under- and Over-Nutrition. Mina Desai, Tie Li, Cristiane Guberman, Michael G. Ross. *Obstetrics & Gynecology, David Geffen School of Medicine at UCLA and Los Angeles Biomedical Research Institute, CA, USA.*

Exposure to either under-nutrition or over-nutrition in early life increases the risk of adult metabolic syndrome. We have shown that both offspring of maternal food restriction and maternal obesity exhibit hypoleptinemia at birth, and hyperphagia and adult obesity. Central hypothalamic appetite regulation involves nutrient/metabolic sensors, (NAD-dependent deacetylase, SIRT1 and mammalian target of rapamycin, mTOR. Both mTOR and SIRT1 colocalize with neuropeptide Y and proopiomelanocortin neurons in the arcuate nucleus. Fasting induced hypothalamic SIRT1 regulation is abnormal in leptin-deficient obese mice, and inhibition/silencing of hypothalamic SIRT1 decreases food intake and body weight gain. Hypothalamic mTOR activity is increased by leptin, and inhibition of mTOR signaling blunts leptin's anorectic effect. Therefore, we determined the hypothalamic expression of SIRT1 and mTOR in FR and HF newborns.

At three weeks of age, female rats were weaned to high fat (HF: 60% k/cal) or (control, 10% k/cal) diet. At 11 weeks of age, these rats were mated and continued on their respective diets during pregnancy. An additional group of dams were 50% food-restricted from pregnancy day 10 to term (FR). Hypothalamic protein expression (Western Blot) was analyzed. Values were normalized to GAPDH and presented as fold change.

At one day of age, FR newborns showed significantly increased hypothalamic SIRT1 expression (1.4-fold) with comparable mTOR expression as the Controls. In contrast, HF newborns had unchanged SIRT1 though significantly decreased mTOR expression (0.6-fold).

The hyperphagia seen in FR and HF newborns is mediated by different metabolic sensor and signaling pathways: In FR newborns upregulated SIRT1, whereas in HF newborns downregulated mTOR, contributes to hyperphagia.

O-2iii-6

Early Weaning Affects the Expression of Hypothalamic Neuropeptide Y (NPY) and Cocaine and Amphetamine-Regulated Transcript (CART) in Adult Rat Offspring. Egberto G. Moura, Viviane Y. Rapozo, Elaine Oliveira, Natalia S. Lima, Alex C. Manhães, Penha C. Barradas, Magna C. Passos, Patricia C. Lisboa. *Physiology, State University of Rio de Janeiro, Rio de Janeiro/Rio de Janeiro, Brazil.*

The interruption of lactation for a short period in the last three days of lactation, with no use of pharmacological substances or maternal separation, causes offspring's malnutrition and hypoleptinemia and programs for metabolic disorders, as higher body weight and adiposity, hyperphagia, hyperleptinemia and central leptin resistance in adult rats. Here, in order to clarify the mechanisms underlying the phenotype observed in adult rats early weaned, we studied the expression of neuropeptide Y (NPY), agouti-related peptide (AGRP), pro-opiomelanocortin (POMC) and cocaine-and amphetamine-regulated transcript (CART) in different hypothalamic nuclei.

In early weaning (EW, n=10) group, lactating rats were involved with a bandage to interrupt lactation in the last three days, while control group (n=10), pups had free access to milk throughout lactation. The offspring were killed at 180 days (1 animal from each litter). Hypothalamic neuropeptides were measured by immunohistochemistry and Western blot.

At 180 days-old, EW offspring showed an increase in NPY staining in hypothalamic nuclei (arcuate, periventricular, paraventricular and lateral hypothalamus), as well as NPY protein content (+68%) in total hypothalamus. CART positive cells of EW offspring had lower immunoreactivity in those same nuclei, associated with a reduction (38%) in its hypothalamic total protein content. AGRP had no changes in staining or Western blot. POMC content was not different; however, its distribution pattern was altered. Control offspring displayed POMC staining located in perinuclear region, while in EW group, POMC staining was distributed in a punctiform pattern.

Our data indicate that precocious weaning can imprint the neuronal circuitry and cause a long term effect on the expression of orexigenic and anorexigenic neuropeptides that can be consequent to the leptin resistance and are coherent with the hyperphagia of these animals.

O-2iv

Integrating Genetic Variants into Cohort Studies to Further Developmental Origins of Health and Disease Research. George Davey Smith. *Invited Speaker, UK.*

O-2iv-1

Maternal Homocysteine Concentrations in Pregnancy and Offspring Birthweight: Epidemiological Associations and Use of Mendelian Randomization, in Two Indian Birth Cohorts. C. S. Yajnik[1], G. R. Chandak[2], C. V. Joglekar[1], D. S. Bhat[1], P. Katre[3], H. Refsum[4], G. Krishnaveni[5], S. Veena[5], C. Fall[6]. *[1]Diabetes Unit, King Edward Memorial Hospital & Research Centre, Pune, India; [2]Centre for Cellular & Molecular Biology, Hyderabad, India; [3]Persistent Systems Ltd, India; [4]Department of Physiology, Anatomy & Genetics, University of Oxford, United Kingdom; [5]Epidemiology Research Unit, Holdsworth Memorial Hospital, Mysore, India; [6]MRC Lifecopurse Epidemiology Unit, University of Southampton, Southampton, United Kingdom.*

Indian subcontinent is the world's capital of low birth weight (LBW), diabetes and cardiovascular disease. Disturbed one carbon metabolism (influenced by dietary folate and vitamin B12) may influence fetal growth. We investigated the causality of the association between maternal tHcy concentrations and offspring birthweight in two Indian birth cohorts using the technique of Mendelian randomization.

We used data from the Pune Maternal Nutrition Study (Pune, India, n=702) and Parthenon Study (Mysore, India, n=526). Maternal demographic and anthropometric characteristics and folate, vitamin B_{12}, and tHcy concentrations were measured at 28 weeks gestation. Offspring birthweight was recorded. Polymorphisms in the *MTHFR* gene (677C→T, 1298A→C) were studied in mothers and babies.

Nineteen percent of mothers were hyperhomocysteinemic (tHcy > 10 mmol/L), 2% had low folate status and 58% had low vitamin B_{12} status (<150

pmol/L). Maternal tHcy concentrations were inversely related to offspring birth weight (standardized β =-0.21, p=0.001), folate concentrations were positively related (standardized β =0.084, p=0.001) while B_{12} concentrations were unrelated. Mothers in highest quintile of tHcy concentrations were 3.4 (CI 2.1, 5.4) times more likely to deliver a LBW baby compared to those in lowest quintile. Maternal *MTHFR* 677C→T genotype predicted higher plasma tHcy concentrations (per allele increase of 0.17SD units, p=0.001) and lower offspring birth weight (per allele decrease of 0.069 SD units, p=0.01). Higher maternal folate concentrations overcame the effect of *MTHFR* 677TT genotype on birth weight. MTHFR 1298A→C polymorphism was not associated with maternal plasma tHcy, or offspring birth weight.
High maternal homocysteine concentrations contribute to fetal growth restriction in India. Improving maternal vitamin B_{12} and folate nutrition may help improve fetal growth, and curtail the epidemic of diabetes and cardiovascular disease.

O-2iv-2

Prevalence and Influence of Perinatal and Early Life Factors for Bronchial Hyperreactivity in Brazilian Children. Nathalia A. Silva[1], Antônio A. Silva[1], Marco C. Barbieri[2], Heloisa Bettiol[2], Raimundo A. Silva[1], Maria L. Figueiredo[1]. *[1]Public Health, Federal University of Maranhão, Maranhão, Brazil; [2]Pediatrics and Childcare, Faculty of Medicine of Ribeirao Preto, São Paulo, Brazil.*
To estimate the prevalence and assess the influence of perinatal and early life risk factors on the occurrence of BHR in children aged seven to 11 years belonging to two Brazilian birth cohorts.
A study was conducted on 658 children based on information collected at birth in Ribeirao Preto (RP), Brazilian Southeast (1994), and in Sao Luis (SL), Brazilian Northeast (1997/1998), and at school age (2004/2005 and 2005/2006). BHR was measured by the metacholine bronchoprovocation test and was considered to be positive when PC20, the concentration responsible for a 20% fall in forced expiratory volume in the 1st second, was <2 mg/mL. Prevalence rates were estimated by Poisson regression in separate models for each city.
Prevalence of BHR was 32,9% in SL and 39,6%. in RP. On the univariate analysis of SL, very low birth weight (IRR=2,47) and children whose mothers had a lower educational level (0-4 years; IRR=1,56) had more risk of BHR. Multivariate analysis showed association only with very low birth weight (IRR=2,24). Univariate analysis in RP, female sex (IRR=0,71) showed a protective effect, whereas parental marital status (IRR=1,29) and atopy (IRR=2,47) were risk factors for BHR. Multivariate analysis in RP showed very high weight at birth (>4250g; IRR=2,49), maternal smoking during pregnancy (IRR=1,52), pneumonia in the first years of life (IRR=1,74) and atopy (IRR=2,61) as risk factors for BHR.
This study had similar prevalences to the ones found in different countries on the ISAACII study. Different variables were associated in each city, showing contrasting profiles when a more developed city was compared with a less developed one in a country of medium income.

O-2iv-3

Investigating the Predictive Adaptive Response Theory in a Brazilian Birth Cohort. Andre K. Portella[1], Patricia P. Silveira[1], Heloisa Betiol[2], Marco Antonio Barbieri[2], Marcelo Z. Goldani[1]. *[1]Nucleo de Estudos da Crianca e do Adolescente - NESCA, Universidade Federal do Rio Grande do Sul - UFRGS, Brazil; [2]Departamento de Puericultura e Pediatria, Universidade de São Paulo - USP, Brazil.*
The Predictive Adaptive Response Theory (PAR) proposes that a mismatch between the early life and adult environment is detrimental to health. To investigate this we analyzed the data obtained from a birth cohort, comparing the programming of glucose homeostasis in individuals from different birth weight categories that also had distinct socio-economic trajectories into adulthood.
We used categories of schooling (high=equal or more than nine years, and low=less than 9 y) to reflect the socio-economic environment (SEE). The individuals were categorized accordingly to the early life SEE, using maternal schooling, and their own adult schooling for the late environment, forming four groups: high-to-high, high-to-low, low-to-high and low-to-low. These groups were then divided according to their birth weight (low birth weight - LBW= lower than 2500g and non-LBW if more or equal to 2500g). We investigated: waist to hip ratio (WHR), homa-index (Homa-IR), and fasting proinsulin (Pro-Ins). For statistical analysis we used a Two-Way ANOVA (environment mobility versus birth weight) split by sex. The significance for p was set to 0.05. A total of 2022 individuals were assessed at 23-24 years in Ribeirão Preto-SP. The category of decreasing SEE, high-to-low, was excluded of the analysis for having only 13 subjects.
In females the WHR was only increased in those low-to-low that were LBW (interaction p=0.015). Despite that, HOMA-IR was increased only in the LBW females from the high-to-high group (interaction p<0.001). Fasting PRO-INS was not affected. In males, the only significant effect was the fasting PRO-INS (interaction p<0.034), which was higher in the high-to-high LBW group.
In this research, the resulting biometric and metabolic phenotype seemed to be differently programmed depending on the interaction of gender, fetal environment, and early versus late environments. The early mismatch of LBW and high SEE was associated with signs of insulin resistance in both genders, whereas the matched LBW-poor environment affected body composition only in females. We therefore assume the PAR theory as very plausible and of extreme health interest, especially in fast changing countries like Brazil.

O-2iv-4

Intrauterine Influences on Insulin Resistance in Prepubertal Children. Oana Maftei[1,2], Melissa J. Whitrow[1,2], Vivienne M. Moore[1,2], Michael J. Davies[1]. *[1]Research Centre for the Early Origins of Health and Disease, Robinson Institute, The University of Adelaide, South Australia, Australia; [2]Discipline of Public Health, The University of Adelaide, South Australia, Australia.*
To examine associations of maternal obesity at conception, glucose intolerance during pregnancy across the entire spectrum, and gestational weight gain with offspring insulin resistance in childhood.
We analysed data from a representative, prospective birth cohort study (Generation 1 Study, n=557) in Adelaide, South Australia, recruited during 1998-2000. At 9.5 years, 443 children (80% of original cohort) took part in the study, 163 providing a fasting blood sample. The main intrauterine exposures were: (1) maternal pre-pregnancy BMI (self-reported pre-pregnancy weight, height measured in early pregnancy); (2) glucose tolerance during pregnancy: normal glucose tolerance (NGT); borderline gestational glucose intolerance (BGGI), defined by a positive oral glucose challenge test (OGCT) and a negative oral glucose tolerance test (OGTT); and gestational diabetes (GD), defined by a positive OGCT and a positive OGTT; (3) gestational weight gain (GWG). The primary outcome was insulin resistance, estimated by homeostasis model assessment (HOMA-IR). Potential confounders were maternal age, parity, smoking, pregnancy-induced hypertension, and education. Current child BMI was considered a potential mediator. Generalized linear models with log link function and Gaussian family were applied to assess the associations.
Child HOMA-IR at 9.5 years was not significantly associated with maternal pre-pregnancy BMI. Exposure to GD was associated with a significantly higher child HOMA-IR relative to offspring of mothers who maintained NGT during pregnancy, robust to confounder adjustment (42.9%, 95%CI 20.9-68.9; p<0.0001), and attenuated by child current BMI z-score adjustment (39.8%, 95%CI 21.9-60.4; p<0.0001). Exposure to BGGI was associated with a reduction in child HOMA-IR after controlling for current BMI z-score (-17.9%, 95%CI -29.9, -3.96; p=0.014). There was no significant association between GWG and child HOMA-IR.
Among the intrauterine factors considered in this study, GD had the strongest association with child HOMA-IR, which was only attenuated by child current BMI. Based on these findings, GD appears to have an independent effect on child insulin resistance before puberty, reinforcing the importance of screening for and management of abnormal glucose tolerance during pregnancy.

O-2iv-5

Standardized Birth Weight Predicts Psychological Well-Being Age 49-51 Years in the Newcastle Thousand Families Study. Kay D. Mann[1], Morven C. Brown[1], John R. Wildman[1], Louise Parker[2], Mark S. Pearce[1]. *[1]Institute of Health and society, Newcastle University, Newcastle Upon Tyne, United Kingdom; [2]Medicine and Pediatircs, Dalhousie University, Canada.*

Early growth has been associated with psychological health in a number of longitudinal follow up studies. However the relative importance of early life associations is often overlooked. The aim of this study was to investigate lifecourse predictors of psychological well being, assessed using the 28-item version of the General Health Questionnaire (GHQ), in the Newcastle Thousand Families Study at age 49-51 yrs.

Detailed information was collected prospectively during childhood, including birth weight, duration breast fed, socio-economic status and childhood IQ. At age 49-51 yrs, 574 study members returned questionnaires including the GHQ-28 and one on health and lifestyle. The four domains (somatic symptoms, anxiety and insomnia, social dysfunction and depression) of the GHQ-28 were collated and a total GHQ score calculated by summing the four domain scores for each individual. A total cut off score of 24 was used to identify 'caseness'. Scores and lifecourse explanatory variables were analysed using linear and logistic regression and path analysis.

After adjustment for all other significant variables, total GHQ score in females was positively associated with birth weight (standardized for sex and gestational age) ($p=0.026$), smoking history ($p=0.04$), and divorced or separated marital status ($p=0.011$) and socio-economic disadvantage ($p=0.05$) at age 49-51 years and negatively associated with sports activity ($p<0.01$) at age 49-51 yrs. Total GHQ score in males was negatively associated with childhood IQ ($p<0.01$) and physical activity ($p=0.029$) at age 49-51 yrs. In females, standardized birth weight was significantly associated with the scores from all four domains and with caseness. Increasing duration breast fed significantly decreased odds for males being cases and was negatively associated with social dysfunction scores. Standardized birth weight had a relatively similar importance compared to factors later in life.

Standardized birth weight was significantly associated with psychological well-being in this cohort, albeit in the opposite direction to that expected. Our findings suggest that both ends of the birth weight spectrum may be of interest and that sex differences should be further investigated.

O-2iv-6

Birth Weight and Cardiovascular Risk Factors at 21 Years in Indians. H. G. Lubree[1], A. J. Pande[1], S. M. Joshi[1], D. S. Bhat[1], P. A Katre[2], D. A. Raut[1], P. S. Hardikar[1], S. V. Kasture[1], L. V. Ramdas[1], A. N. Pandit[1], C. H.D. Fall[3], C. S. Yajnik[1]. *[1]Kamalnayan Bajaj Diabetology Research Centre and Paediatrics Department, KEM Hospital Research Centre, Pune, India; [2]Persistent Systems P. Ltd, Pune, India; [3]MRC Lifecourse Epidemiology Unit, Southampton, United Kingdom.*

Pune Children's Study (PCS) was established to prospectively examine early-life antecedents of adult disease. At 8y of age, low birth weight and high current weight were associated with higher insulin resistance and CVD risk. We now report associations of birth weight and current weight with CVD risk factors at 21 years of age.

We studied 351 children at 21 years of age. We measured OGTT (75g glucose, WHO 1999), lipids, blood pressure and anthropometry. Plasma glucose, insulin (fasting, 30, 120 min) concentrations were measured. Insulin resistance was calculated by HOMA-IR and sensitivity by Matsuda index.

Three children with type-1 were excluded from analysis. There were 185 males and 163 females. Children were taller than their expected height calculated from the mid-parental height (males 5.8 and females 4.1 cms), BMI was 21.7 kg/m2. Nineteen percent were overweight and 3% were obese (BMI>30). Twenty seven were hyperglycemic (1 IFG, 21 IGT, 5-DM). Twenty had hypercholesterolemia (>200 mg%), 25 had hypertriglyceridemia (>150 mg%), 193 had low HDL cholesterol (males <40, females <50 mg%) and 15 were hypertensive (BP 130/85 mmHg). The hyperglycemic children had higher Waist-hip ratio and sum of skinfolds at 8y while were heavier, taller, had higher BMI and adiposity, cholesterol, triglycerides and blood pressure at 21 y as compared to normoglycemic children ($p<0.05$ for all). LBW Children (<2.5 kg) were lighter, shorter, had higher pulse rate and lower insulin sensitivity at 21y as compared to others. Using multiple linear regression analysis (Lucas model), low birth weight predicted higher pulse and lower insulin sensitivity ($p<0.05$ for all). Children with lower birth weight and higher current weight had higher 120 min glucose, blood pressure, triglycerides, insulin resistance ($p<0.05$ for all). Current weight predicted all risk factors. Interaction between birth weight and current weight was not significant. All the above results are adjusted for gender.

Lower birth weight and higher adult weight contribute to higher metabolic and cardiovascular risk in the Pune Children Study.

O-2v-1

Maternal Protein Restriction Decreases Global Histone H3 K27 Methylation in the Liver of Adult Offspring through the Altered Expression of Specific microRNAs. Karen A. Lillycrop, Abigail Lapham, Danielle O'Neil, Anita Gola, Mark A. Hanson, Graham C. Burdge. *Institute of Developmental Sciences, University of Southampton, Hampshire, United Kingdom.*

MicroRNAs are short non-coding RNAs which play a key role in regulating gene expression(1). Maternal nutritional constraint is associated with long-term effects on the metabolism and physiology of the offspring(2), but it is not known whether these effects involve altered expression of microRNAs. We, therefore, investigated in rats the effect of maternal protein-restriction (PR) on the expression of hepatic microRNAs in adult offspring.

Rats were fed either a control (18% (w/w) casein) or PR diet (9% (w/w) casein) from conception to delivery, then standard chow (AIN-76A) during lactation. Male offspring were weaned onto a semi-purified diet and killed on PN84. Liver RNA from six control and six PR offspring were pooled and hybridized in duplicate to an Agilent G4473A miRNA microarray. Microarray analysis was confirmed for specific miRNA by real time PCR. Global H3 K27 methylation was measured using Elisa.

The expression of six microRNAs differed significantly ($\geq$ 1.5-fold change, $P<0.05$) between offspring of PR and control dams. miR-30c2 and miR-101 which exhibited the largest difference in expression, target components of the polycomb complex, eed and EZH2, which regulate epigenetic developmental processes through methylation of lysine 27 on histone H3(3). Increased miR-30c2 and 101 expression in the liver of PR offspring was accompanied by a decrease in the expression of their target mRNAs, eed and EZH2, and a decrease in the global level of H3 K27 methylation in the liver of PR compared to control offspring.

The polycomb complex plays a pivotal role in maintaining gene expression patterns of different cell types by regulating both histone and DNA methylation(4) These data suggest that altered expression and activity of the polycomb complex may be one important mechanism by which early life environment could induce changes in the programme of gene expression during development and so alter the phenotype of the offspring.

MAH receives salary support from the British Heart Foundation.

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O-2v-2

The Intensity of IUGR-Induced Transcriptome Deregulations Is Inversely Correlated with the Onset of Organ Function in a Rat Model. Daniel D.V. Vaiman[1,2], Geraldine G.G.L. Gasmoin-Lachambre[1,2], Farid F.B. Boubred[3,4], Isabelle I.L. Ligi[3,4], Francoise F.M. Mondon[1,2], Jean-Marc J.M.F. Feuerstein[5], Isabelle I.G. Grandvuillemin[3,4], Umberto U.S. Simeoni[3,4], Christophe C.B. Buffat[6]. *[1]Génomique, Epigénétique et Physiopathologie de la Reproduction, INSERM, Institut Cochin, France; [2]Université Paris Descartes, France; [3]Service de Médecine Néonatale - Hôpital Conception, Assistance Publique Hôpitaux de Marseille, France; [4]UMR 6008, INSERM, Université de la Méditerranée, France; [5]Centre de formation et de recherches expérimentales médico-chirurgicales, Faculté de Médecine Marseille, Université de la Méditérannée, France; [6]Laboratoire de Biologie Moléculaire - Hôpital Conception, Assistance Publique Hôpitaux de Marseille, France.*

A low-protein diet applied during pregnancy in the rat results in intrauterine growth restricted (IUGR) fetuses. In humans, IUGR is an important clinical problem associated with increased perinatal morbidity, higher incidence

of neuro-developmental defects and increased risk of adult metabolic anomalies. It is now clear that development and later function of many organs can be affected by environmental conditions. Very few studies have investigated at the same time several organs, on a more comparative basis. However, it is quite probable that IUGR affects differentially most organ systems, with possible persistent changes in gene expression.
In this study we address transcriptional alterations induced by IUGR in a multi-organ perspective.
We show that (1)expressional alterations are apparently stronger in organs functioning late in foetal or postnatal life than in organs that are functioning early (2)hierarchical classification of the deregulations put together kidney and placenta in one cluster, liver, lungs and heart in another; (3)the epigenetic machinery is set up especially in the placenta, while its alterations are rather mild in other organs; (4)the genes appear deregulated in chromosome clusters; (5)the altered expression cascades varies from organ to organ,(6) we found a significant increase in TF binding site for HNF4 proteins specifically for liver genes that are down-regulated in IUGR, suggesting that this decrease is achieved through their action.
Our study suggests that a combination of tissue-specific mechanisms contributes to bring about tissue-driven modifications of gene cascades. The question of these cascades being activated to adapt the organ to harsh environmental condition, or as an endpoint consequence is still raised.

O-2v-3

Maternal Obesity Influences Hepatic Gene Expression and Genome-Wide DNA Methylation in Offspring Liver at Weaning. Kartik Shankar[1,2], Sarah J. Borengasser[1,2], Ying Zhong[1], Horacio Gomez-Acevedo[1,2], Martin J. Ronis[1,3], Thomas M. Badger[1,2]. *[1]Arkansas Children's Nutrition Center, AR, USA; [2]Pediatrics, University of Arkansas for Med Sci., AR, USA; [3]Pharm & Tox, University of Arkansas for Med Sci., AR, USA.*
Offspring from obese rat dams gain greater body weight and fat mass when fed HFD. Here we examine hepatic gene expression related to systemic energy expenditure and alterations in genome-wide DNA methylation.
Maternal obesity was produced in rats prior to conception via overfeeding of diets. At PND21, we examined energy expenditure (indirect calorimetry), hepatic gene expression (microarrays), and changes in genome-wide DNA methylation (enrichment-coupled sequencing, Illumina), coupled with specific signaling pathway analysis.
Microarray analyses at PND 21 revealed a reprogramming of lipogenic and lipid degradative pathways. This was associated with increased expression of SREBP-1 and 20 lipogenic effectors, and decreased PPAR-α/AMPK signaling. Offspring from obese dams had decreased energy expenditure ($p<0.05$) and higher respiratory exchange ratio values ($p<0.05$) on either AIN-93G or HFD, indicating an impaired capacity to utilize fatty acids(FA). Mitochondrial protein content of electron transport chain complexes (II, III, and ATPase) was decreased ($p<0.03$) in offspring from obese dams. Hepatic mRNA and mitochondrial protein expression of SIRT3, an critical regulator of mitochondrial oxidative capacity and FA oxidation were decreased ($p<0.002$) in offspring from obese dams. Fasting-induced increases in mRNA and protein expression of PGC-1α and PPAR-α were significantly diminished in offspring of obese dams. Finally, we employed a procedure to enrich methylated DNA using capture with methylbinding protein followed by Illumina sequencing. MACS analysis identified more than 4,000 differentially methylated regions. Following classification of the regions based on CpG content (cut-off > 3%), and ±5 Kb window of annotation, 799 genes were identified with differentially methylated regions. Based on this analysis, the 3'-UTR region of PPAR-α was found to be hypermethylated in offspring of obese dams consistent with impaired fatty acid mobilization.
Maternal obesity influences early hepatic gene expression and DNA methylation. Further it appears that mitochondrial dysfunction precedes the development of energy utilization perturbations, hepatic steatosis and adiposity in offspring from obese rat dams.

O-2v-4

Hepatic Epigenetic Changes in MEG3 and IGF2R Imprinted Genes in Adult Sheep Following Prenatal and Postnatal Undernutrition. Robert J. Murray[1], Kirsten R. Poore[1], Jane K. Cleal[1], Keith M. Godfrey[2,3], Mark A. Hanson[1,3], Graham C. Burdge[1], Lucy R. Green[1], Karen A. Lillycrop[1]. *[1]Institute of Developmental Sciences, United Kingdom; [2]MRC Lifecourse Epidemiology Unit, United Kingdom; [3]NIHR Nutrition Biomedical Research Unit, University of Southampton, United Kingdom.*
Prenatal and postnatal undernutrition have sex-specific effects on growth, metabolism and cardiovascular control in mature adult sheep offspring (Cleal *et al.*. 2007 PNAS 104, 9529; Poore *et al.* 2007 AmJPhys. 292, E32; 2010 JPhys. 588, 2219). We investigated an epigenetic basis for these sex-specific effects by measuring DNA methylation at imprint control regions of the *Igf2r* and *Dlk1*/*Meg3* genes, thought to be involved in adipocyte development and growth.
Welsh mountain ewes received 100% (C, n=34) or 50% of total nutrient requirements (U, n=39) from 1-31 days of gestation, and 100% thereafter. Offspring were fed *ad libitum* (CC, n=20; UC, n=17) or to reduce body weight to 85% of individual target weight from 12 to 25 weeks postnatal age and *ad libitum* thereafter (CU, n=14; UU, n=22). Offspring were killed at 2.5 years. DNA was extracted from left lobe of the liver and bisulphite converted. PCR was used to amplify portions of the *Igf2r* gene (within intron 2) and the *Meg3* gene (upstream of the transcriptional start site and containing a CTCF consensus sequence, thought to regulate imprinting at the *Dlk1*/*Meg3* imprinted cluster). Methylation of individual CpG dinucleotides was determined by pyrosequencing. Data were analyzed by ANOVA.
Methylation was decreased at three CpG dinucleotides within the predicted CTCF site of the ICR at the *Dlk1*/*Meg3* imprinted cluster ($P<0.01$) and at the CpG site immediately downstream ($P<0.05$) in females following postnatal undernutrition, regardless of prenatal nutrition. Methylation was increased at three CpG dinucleotides within the ICR at the *Igf2r* imprinted region ($P<0.03$) in males following prenatal undernutrition, regardless of postnatal nutrition.
This is the first study to show that methylation of ICRs of imprinted gene clusters *Dlk1*/*Meg3 and Igf2r* is altered by prenatal and postnatal undernutrition in adult sheep. These epigenetic control changes are sex-specific and, given the key roles of these genes in adipocyte development and growth, they may contribute to the sex-specific changes in metabolism and growth observed in this model.
Supported by British Heart Foundation, University of Southampton, UK Medical Research Council and EpiGen consortium.

O-2v-5

Identification and Characterization of Human Metastable Epialleles. Wenjuan Zhang[1], Eleonora Laritsky[1], Maria S. Baker[1], Lanlan Shen[1], Robert A. Waterland[1,2]. *[1]Department of Pediatrics, Baylor College of Medicine, USDA/ARS Children's Nutrition Research Center, TX, USA; [2]Department of Molecular & Human Genetics, Baylor College of Medicine, TX, USA.*
Maternal nutrition before and during pregnancy affects the establishment of DNA methylation at murine metastable epialleles (MEs) such as *agouti viable yellow* and *axin fused*, causing permanent phenotypic changes . Following on the success of our recent small scale screen for human MEs, we have developed an approach combining a broader genome-wide screen with a machine learning algorithm to both identify more human MEs and better understand epigenetic metastability.
Using human postmortem tissues representing the three embryonic germ layers: liver (endodermal), kidney (mesodermal) and brain (ectodermal), we identified systemic interindividual variation in DNA methylation by methylation-specific amplification microarray (MSAM). Genomic intervals showing concordant interindividual variation in all three tissues were considered candidate MEs. Systemic interindividual DNA methylation differences were validated by bisulfite pyrosequencing. Based on bioinformatically annotated associations of MEs with various genomic features, we developed a machine learning algorithm to predict novel MEs in the human genome.
The MSAM screen identified 278 intervals as candidate MEs. Bisulfite pyrosequencing confirmed seven of nine as *bona fide* MEs. 35% of MEs were located in introns, vs. 15% of non-ME control intervals ($p<10^{-36}$). Repetitive elements including long terminal repeat retrotransposons,

simple tandem repeats, DNA transposons, and SINEs were enriched near MEs. Conversely, ME regions were relatively depleted of simple and low-complexity repeats and CpG islands ($p<10^{-170}$ for each MEs vs control comparison). We identified 11 50-100 bp motifs dramatically enriched near MEs ($p<10^{-84}$). After 10-fold cross-validation training, a support vector classification algorithm provided simulated prediction accuracy of 97.5%, with only a 0.6% false positive rate. Results of applying this algorithm for genome-scale ME identification will be reported.

We anticipate that our machine learning algorithm will enable the identification of many more human MEs than are detectable by MSAM. Given the systemic interindividual epigenetic variation at these loci, MEs identified near genes associated with human disease will provide attractive candidate loci at which to explore the epigenetic basis of the developmental origins hypothesis.

O-2v-6

Histone Deacetylase SIRT1 Alterations Parallel Fetal Epigenetic and Metabolic Reprogramming in Response to a Maternal High Fat (MHF) Diet. Kjersti Aagaard[1], Melissa Suter[1], Aishe Chen[1], Lori Showalter[1], Cindy Shope[1], Robert Lane[2], Kevin Grove[3]. *[1]Obstetrics&Gynecology, Baylor College of Medicine, USA; [2]Pediatrics, University of Utah, USA; [3]Oregon National Primate Research Center, USA.*

Our prior studies in primates demonstrate that a maternal high fat diet (rather than obesity) induces fetal nonalcoholic fatty liver (NAFLD) and alters the epigenome and metabolome. These changes are accompanied by epigenetically altered acetylation of histone H3 at lysine 14 (H3K14ac). However, the responsible histone deacetylase has heretofor eluded us. As SIRT1 is both a lysine protein deacetylase and a crucial mediator of longevity following caloric restriction, we hypothesized that SIRT1 may also be a **histone** deacetylase integral to modulating the fetal epigenome.

Pregnant macaques were fed control (*n*12) or HF (*n*19) diet, with a subset reverted to control diet in later years (*n*7). Serum and hepatic tissue was obtained from e130 offspring. Acid extracted histones, DNA, RNA, and protein were employed for quantitation and significance was determined with 2-tailed T-tests, Fischer´s Z transformation, and ANOVA as appropriate. In order to directly demonstrate SIRT1 histone deactylase activity, TOLDI mass spectrometry (MS) against engineered H3 modification-specific peptides was employed in native material and transfected Cos-1 cells.

High fat diet exposure increased fetal H3K14ac (1.9 fold $p<.01$) with concomittant decreased SIRT1 expression (0.86 fold, $p=.03$) and accompanied diminished in vitro HDAC activity (0.81, $p<.05$). Despite peristent maternal obesity, altered fetal H3K14ac and SIRT1 are abrogated with diet reversal (0.94, $p<.01$ and 0.95, $p=0.07$, respectively). Direct observations of purified Sirt1-mediated H3K14 deacetylation by MS is observed (-42 Da peak shift); this is abrogated with transfection of a dominant-negative mutant SIRT1. Finally, known downstream effectors dysregulated in NAFLD and modulated by SIRT1 are observed to increase (*i.e.*, SREBP1 2.4-fold, PPARγ 1.83-fold; $p<.01$).

Altered fetal expression of the deacetylase SIRT1 following *in utero* exposure to a MHF diet (or reversion thereof) parallels modulation of H3K14ac, HDAC activity, FFA & lipids, and their biologically-relevant transcription factors (SREBP, PGC1α, PPARγ). Our data demonstrates that SIRT1 is both a protein and a histone deacetylase, and implicates it as a likely molecular mediator of the primate fetal epigenome and metabolome.

O-2v-7

Chronic Hypoxia In Utero (CHU) Induces Increases in Sympathetic Innervation and Activity That Exist into Early Adulthood. William Rook, Andrew M. Coney, Janice M. Marshall. *CEM-Physiology, University of Birmingham, West Midlands, United Kingdom.*

Sub-optimal conditions in utero are associated with intrauterine growth restriction (IUGR) and increased risk of adult cardiovascular disease is well established - CHU is a well-known cause of IUGR. In the chronically hypoxic chick egg (d19 fetus), the femoral artery (which feeds hindlimb muscle) showed increased sympathetic nerve density and increased noradrenaline content [Rouwet, 2002; Ruijtenbeek, 2000] and at 14-15 weeks old, an exaggerated sympathetic vasoconstriction [Ruijtenbeek, 2003], however, no recordings of ongoing muscle sympathetic nerve activity (MSNA) were made. Further, whether similar hyperinnervation occurs in mammals and persists into adult life is not known, nor is whether there are changes in ongoing MSNA. We have therefore investigated these possibilities.

Experiments were performed in age-matched male (10-12wk old) normal (N) and CHU Wistar rats. CHU rats were the offspring of pregnant Wistar dams who were exposed to 12% O_2 from day 10-20 of pregnancy and then returned to air to give birth and raise the litter. Recordings of MSNA were made using the focal recording technique on the exteriorized spinotrapezius muscle [Hudson, 2011] under Alfaxan anaesthesia (10-15mg.kg^{-1}.hr^{-1}); arterial blood pressure (ABP) and tracheal pressure (for respiratory rate) were also monitored. After killing by anaesthetic overdose, tibial arteries (5mm lengths) were taken and mounted as stretch preparations and the sympathetic nerve fibres were stained using the glyoxilic acid staining technique [Furness, 1975]. They were viewed using confocal microscopy and quantitative analysis. Comparisons between N and CHU were made by Student's unpaired t-test.

Both N and CHU rats had similar baseline ABP, femoral vascular resistance and respiratory rate [Coney, 2010], however, ongoing MSNA was significantly higher in CHU rats (0.56±0.075Hz vs 0.33±0.036Hz in N; $p=0.003$). Fluorescent area in tibial arteries (more than 3SDs above mean background intensity) was higher in CHU than N rats (N: 10.5±0.72% (n=10), CHU: 16.4±1.8% (n=13), $p=0.012$). Sympathetic nerve density was also markedly higher in CHU (N: 0.75±0.029, CHU: 1.09±0.15 intersects/unit area, $p<0.001$).

Adult CHU rats have higher levels of ongoing MSNA, and hyperinnervation of the tibial artery (that supplies muscles of the hindlimb). These do not translate into changes in muscle vascular resistance or gross systemic cardiovascular consequences at this age.

O-2vi-1

Fat Content of Maternal Diet Alters Female Offspring Fatty Acid Status through Epigenetic Regulation of *Fads2*. Samuel P. Hoile[1], Nicola A. Irvine[1], Christopher K. Kelsall[1], Aurelie Feunteun[1], Karen A. Lillycrop[2], Christopher Torrens[1], Philip C. Calder[1], Mark A. Hanson[1], Graham C. Burdge[1]. *[1]Faculty of Medicine, University of Southampton, Hampshire, United Kingdom; [2]Faculty of Natural and Environmental Sciences, University of Southampton, Hampshire, United Kingdom.*

In rodents, feeding a high saturated fat diet during pregnancy and lactation induces phenotypic changes in offspring (Armitage 05). It is not known whether type or amount of dietary fat influences this and the mechanism that underlies such effects is unknown. Maternal nutritional exposure including global under nutrition induces phenotypic changes in offspring through epigenetic regulation of specific genes (Burdge & Lillycrop 10). We investigated the effect of the amount and type of maternal dietary fat on the epigenetic regulation of hepatic polyunsaturated fatty acid (PUFA) metabolism in adult female offspring.

Dams were fed diets containing n-6 PUFA (safflower oil, SAO), *trans* fatty acids (hydrogenated soybean oil, HSO), saturated fatty acid (butter) or 20:5n-3 and 22:6n-3 (fish oil, FO) at either 7%(w/w) or 21%(w/w) from conception until offspring were weaned onto AIN93G containing 4%(w/w) soybean oil. Liver and blood were collected on day 77. Liver and plasma phosphatidylcholine (PC) PUFA composition was measured by gas chromatography. mRNA expression of *Fads2*, which encodes Δ6 desaturase, which is rate limiting in the PUFA desaturation/elongation pathway, was measured by real time RTPCR. CpG methylation in the *Fads2* promoter was measured by pyrosequencing.

21% maternal dietary fat reduced proportions of liver and plasma arachidonic acid (AA) and docosahexanoic acid (DHA) ($P<0.05$) irrespective of fat type. *Fads2* mRNA was lower in offspring of dams fed 21% vs. 7% fat and was positively associated ($P<0.005$) with levels of AA and DHA in liver and plasma PC. There were effects of fat type ($P<0.0001$) on methylation at CpGs -623, -394, -84 and -76. Methylation of CpG -394 was negatively correlated with the level of liver and plasma PC AA and DHA ($P<0.001$).

The data suggests that maternal fat intake induces persistent changes in AA and DHA biosynthesis in female offspring through altered epigenetic regulation of *Fads2*. This may induce reduced capacity for PUFA synthesis in offspring and in turn, incorporation of alternative fatty acids in phospholipids of cell membranes. This will result in altered membrane structure and fluidity, affecting protein interactions and response to stimuli.

O-2vi-2

Increased Nocturnal Lipid Oxidation in Young Men Who Had Low Birth Weight. Charlotte Brøns[1,2], Søren Kruse Lilleøre[3], Christine Bjørn Jensen[3], Søren Toubro[4], Allan Vaag[1,2], Arne Astrup[4]. *[1]Diabetes and Metabolism, Rigshospitalet, Copenhagen, Denmark; [2]Steno Diabetes Center, Denmark; [3]Novo Nordisk, Denmark; [4]University of Copenhagen, Denmark.*

Low birth weight (LBW), indicating an adverse fetal environment, is associated with increased risk of type 2 diabetes (T2D). Previous studies have documented a range of early metabolic defects in young and healthy men born with LBW including abdominal obesity, mildly elevated fasting blood glucose levels, as well as liver and muscle insulin resistance. Altered energy expenditure (EE) and an increased lipid oxidation rate are common features of the overt T2D phenotype, although the origin of these metabolic dysfunctions as well as their exact role(s) in the development of T2D remains controversial. Using respiratory chambers, we aimed to investigate whether young and healthy LBW men with a known increased risk of T2D exhibit altered 24-hours EE and/or fat oxidation rates prior to the development of T2D, potentially representing primary metabolic defects due to altered developmental programming.

Forty-six young, healthy and lean men matched for age and BMI were included in the study. Twenty LBW subjects had a birth weight below the 10th percentile for gestational age (2688 ± 269 g) and 26 control subjects had a normal birth weight (NBW) between the 50th and 90th percentile (3893 ± 207 g) ($P < 0.0001$). Subjects were fed a standardized weight maintenance diet and 24-h EE, respiratory quotient (RQ), and substrate oxidation rates were assessed using a respiratory chamber.

No differences in overall average 24-h EE, RQ or substrate oxidation rates were observed between LBW and NBW subjects. Nevertheless, the LBW group exhibited significantly lower mean adjusted RQ during sleep compared to NBW controls (0.81 ± 0.01 vs. 0.85 ± 0.01, $P = 0.01$), and with no difference in nocturnal EE between groups, the LBW subjects displayed a significantly elevated rate of nocturnal fat oxidation compared with the NBW controls (2.55 ± 0.13 kJ/min vs. 2.09 ± 0.12 kJ/min, $P = 0.02$).

In conclusion, an increased rate of nocturnal lipid oxidation is an early defect of metabolism in subjects born with LBW, potentially increasing the level of fasting glucose and risk of T2D via the induction of hepatic insulin resistance.

O-2vi-3

Chronic Mild Pulsatile Hyperglycemia Suppresses Glucose-Stimulated Insulin Secretion and Increases Accumulation of Reactive Oxygen Species in Fetal Sheep Islets. Alice S. Green[1], Xiaochuan Chen[1], Antoni R. Macko[1], Miranda J. Anderson[1], Amy C. Kelly[1], Ronald M. Lynch[2], Sean W. Limesand[1]. *[1]Animal Sciences, University of Arizona, AZ, USA; [2]Departments of Pharmacology and Physiology, University of Arizona, AZ, USA.*

Children from diabetic pregnancies have a higher incidence of developing Type 2 diabetes, and fetal exposure to hyperglycemia is a risk factor for islet dysfunction. β-cell responsiveness was examined after a two week exposure to mild sustained hyperglycemia with repeated pulses in fetal sheep.

Beginning at 0.84 of term, mild pulsatile hyperglycemia (mPHG, n=5) and pulsatile hyperglycemia ewes (PHG, n=10) received two dextrose infusions: a constant infusion that raised plasma glucose concentrations by 15% or 20% and 45-min boluses at 0800, 1400 and 2000 h each day that raised glucose by 55% or 100%, respectively. Control ewes (n=10) received saline.

Fetal glucose-stimulated insulin secretion (GSIS) and glucose potentiated arginine insulin secretion (GPAIS) were lower (P≤0.05) in PHG fetuses with plasma insulin concentrations at 0.86±0.13 and 2.91±0.39 ng/ml compared to controls (1.58±0.25 and 4.51±0.56 ng/ml), and mPHG fetuses were intermediate (1.21±0.08 and 4.25±0.56 ng/ml). In isolated islets, stimulatory glucose concentrations (11.1 mmol/L) increased (P<0.05) insulin secretion (1.6 fold) and glucose oxidation rates (2 fold), but treatments were not different. Islet insulin content was 35% lower in PHG and 35% higher in mPHG compared to controls (P<0.01). Also, isolated islets loaded with CM-H2DCFDA (Invitrogen), a fluorescent ROS probe, had greater ROS accumulation in the presence of 11.1 mmol/l glucose; the ratio of stimulated/basal rates was 1.6±0.2 for PHG islets vs. 0.9±0.1 in control islets (P<0.01). Evidence for islet protein oxidative damage was not observed, but PHG treatment increased oxidative damage to skeletal muscle proteins (P<0.05).

These findings indicate that pulsatile hyperglycemia, mimicking that of diabetic mothers, leads to impaired insulin secretion responsiveness and dysregulation of islet ROS production.

O-2vi-4

Breastfeeding and Metabolic Risk Reduction at the Time of Lactation: The Women and Their Children's Health (WATCH) Cohort. Alexis Hure[1,2], Clare Collins[1,3], Julia Martin[1,2], Roger Smith[1,3]. *[1]Mothers & Babies Research Centre, Hunter Medical Research Institute, NSW, Australia; [2]School of Medicine & Public Health, University of Newcastle, NSW, Australia; [3]School of Health Sciences, University of Newcastle, NSW, Australia.*

To quantify the differences in known cardiovascular and diabetic risk markers in women up to six months after birth, according to their lactation status and postpartum weight loss.

Pregnant women (n=180) were recruited to the WATCH Study by research midwives in the antenatal clinic of the John Hunter Hospital, Newcastle. Maternal weight data and blood samples were collected at approximately 20 and 36 weeks gestation during pregnancy, and then at three and six months postpartum, after a 12 hour fast. Blood samples were assayed for lipids and markers of glycaemia immediately after sample collection. Infant feeding data were recorded at three and six months postpartum using prospective and recall methods administered by the Research Dietitian. Metabolic risk markers according to percentage weight loss since 36 weeks gestation (≥14% or <14%) and breastfeeding status at six months after birth (any or none), were compared using t-tests (unequal variance), excluding those with diagnosed gestational or pre-existing diabetes. Measured potential confounders also include maternal age, parity, BMI, gestational weight gain, breastfeeding exclusivity, education, income and smoking.

At six months after birth:

Women reporting any breastfeeding (N=66) with a weight loss of ≥14% since 36 weeks gestation (n=39) had significantly lower fasting total cholesterol, triglycerides, LDL-C, HDL-C/total cholesterol, and glucose (P<0.05) compared to those who had lost <14% weight (n=27). For those not breastfeeding (N=50), HDL-C was significantly higher (P=0.03) and HDL-C/total cholesterol, insulin, and HOMA-IR were significantly lower (P≤0.02) in those with ≥14% weight loss (n=21) compared to <14% weight loss (n=29). When postpartum weight loss was ≥14% (N=60) breastfeeding women (n=39) had significantly lower triglycerides, glucose, HOMA-IR, and HbA1c (P≤0.04) and higher HDL-C (P=0.01) vs. no breastfeeding (n=21).

Breastfeeding women had significantly better cardiovascular and diabetic risk profiles at the time of lactation than women not breastfeeding, even after considering postpartum weight loss, a major confounding factor. These results may help to explain the reduced prevalence of cardiovascular disease and diabetes in postmenopausal women with a history of lactation.

O-2vi-5

Postnatal Diet, but Not Maternal Obesity Is Associated with Sex-Specific Effects on Offspring Metabolism. V. King[1], J. E. Norman[1], J. R. Seckl[2], A. J. Drake[2]. *[1]Tommy's Centre, University of Edinburgh, United Kingdom; [2]Centre for Cardiovascular Science, University of Edinburgh, United Kingdom.*

Maternal obesity increases offspring obesity and cardiovascular disease risk; however it is unclear to what extent this is a consequence of 'programming' *in utero* or the effect of postnatal diet. Here, we have investigated the effects of maternal obesity and postnatal diet on offspring metabolism in C57Bl/6 mice.

Female mice were maintained on obesogenic (OB, 58% kcal fat, 25.5% kcal carbohydrate as sucrose) or control diets from five weeks. Females were mated at 17 weeks, remaining on the same diets through pregnancy and lactation. Offspring of obese (DIO) and control (CON) mothers were weaned onto OB or control diets to generate four groups (C/C, C/D, D/C, and D/D). At six months, offspring underwent glucose tolerance tests (GTT, n=7-8/group). Data were analysed by 2-way ANOVA.

Females on OB diet became hyperinsulinaemic and hyperlipidaemic, and were heavier than controls before and during pregnancy, however there were no differences in offspring birth weights (CON 1.32±0.01 vDIO 1.27±0.02g; NS). At weaning, DIO offspring were heavier than CON (CON 8.2±0.16 vDIO 10.9±0.16g, p<0.001). Postnatal OB diet resulted in increased weight

gain (male C/C 28.1±1.1, C/D 46.1±2.1, D/C 29.5±0.5, D/D 41.3±1.9g; p<0.001; female C/C 22.3±0.4, D/C 28.9±1.4, D/C 20.6±0.6, D/D 29.8±0.5g; p<0.001). Male offspring on OB diet were hyperglycaemic (AUC male C/C 1189±124, C/D 2287±280, D/C 1205±163, D/D 2074±143 mmol/l.min; p<0.01) and hyperinsulinaemic (AUC male C/C 108±16.8, C/D 581±61, D/C 129±14, D/D 535±52ng/ml.min; p<0.001) on GTT with increased plasma cholesterol (male C/C 2.8±0.4, C/D 9.4±0.5, D/C 4.1±0.3, D/D 7.8±0.5mmol/l; p<0.001). Female offspring on OB diet were moderately hyperinsulinaemic (AUC C/C 58.5±9.7, C/D 123.7±22.3, D/C 49±4.7, D/D 153±27.1ng/ml.min; p<0.001) but remained normoglycaemic on GTT. There were no effects of prenatal diet on any parameter in either sex. No differences were found in liver weight; however postnatal OB diet reduced kidney weight in both sexes, regardless of *in utero* environment (p<0.01). Thus, exposure to an obesogenic diet after weaning is associated with sex-specific effects on metabolism with females relatively protected from the adverse metabolic consequences of obesity. Exposure to an 'obesogenic environment' during gestation and lactation does not inevitably result in the programming of obesity or overt metabolic effects in adult offspring.

O-2vi-6

Maternal High Fat Diet Alters Milk Composition, Offspring Metabolism and Adrenal Function at Weaning. Juliana G. Franco[1], Isis H. Trevenzoli[2], Tatiana P. Fernandes[2], Camila P. Dias-Rocha[2], Luana M. Pasqualinni[2], Carmen C. Pazos-Moura[2], Patrícia C. Lisboa[1], Magna C.F. Passos[1], Egberto G. Moura[1]. *[1]State University of Rio de Janeiro, Rio de Janeiro, Brazil; [2]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.*

Increasing evidence suggests that maternal obesity in critical periods (gestation and lactation) is important in promoting obesity in offspring. Here, we evaluated the effect of maternal high fat intake on milk composition and its impact upon leptinaemia, energy metabolism and adrenal medullary function of the offspring at weaning in a model of programming by maternal obesity.

Wistar female rats were fed with normal (9% fat; C group) or high fat diet (29% fat; HF group) for eight weeks before matting and during pregnancy and lactation. Body composition was evaluated by DEXA. Glycemia, liver glycogen, milk composition, serum and milk leptin concentration and adrenal cathecolamine content were evaluated. Differences were considered significant at p<0.05.

HF mothers presented higher total fat body content after eight weeks (+27%) and similar fat content at the end of lactation. Milk from HF group had higher content of lactose (+20%) and lower content of cholesterol (-19%) in the 11th day of lactation. At weaning, milk had higher content of proteins (+18%) and cholesterol (+52%). Differences in leptin milk concentration were not observed. Offspring from HF mothers presented higher BW (+53%) with increase in fat depots: retroperitoneal (2.3 fold), epididymal (3.4 fold) and inguinal (2 fold) and higher glycemia (+30%), leptinaemia (+62%), adrenal catecholamine (+17%) and liver glycogen (+50%) at weaning.

HF diet increases maternal body fat and this additional energy seems to be transferred to the offspring during gestation and lactation, since at weaning dams showed normal fat and pups displayed obesity. The higher content of cholesterol and protein in the milk at weaning seems to induce early overnutrition. Leptin may stimulate adrenal medullary function and HF offspring showed hyperleptinaemia and higher catecholamine content, which may contribute to the programming of cardiovascular disease. Besides store energy as fat, HF offspring present larger reserve of glycogen and hyperglycemia that may be a consequence of a preferential use of fat as energy source. Several of these reported changes in offspring, such as hyperglycemia, hyperleptinemia and changes in the production of catecholamines, can act as imprinting factors for future programming.

O-2vi-7

Parenteral Nutrition and Altered Omega-3 Fatty Acid Profiles in the Extremely Preterm Infant: First Steps in Programmed Inflammatory Responses and Oxidative Stress? Michael J. Stark[1,3], Frederike Bekker[3], Nicolette A. Hodyl[1], Andrew J. McPhee[3], Carmel Collins[2], Robert A. Gibson[2], Maria Makrides[2]. *[1]The Robinson Institute, University of Adelaide, South Australia, Australia; [2]Child and Nutrition Research Centre, University of Adelaide, South Australia, Australia; [3]Perinatal Medicine, Women's and Children's Hospital Adelaide, South Australia, Australia.*

The omega-3 fatty acids docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) are associated with anti-inflammatory and anti-oxidant actions in pregnancy, the neonatal period and protective actions against obesity and allergic disease in later life. The accumulation of omega-3 fatty acids in the fetus is greatest in the last trimester of pregnancy. For infants born extremely preterm parenteral nutrition is essential for growth and nutrition but contains little of the EPA and DHA precursor α-linoleic acid. This study measured erythrocyte membrane fatty acid profiles and measures of oxidative stress in the first weeks of neonatal life.

Free fatty acid profiles derived from erythrocyte membrane phospholipids were quantified by capillary gas chromatography in infants born less than 28 weeks gestation (n=20) administered parenteral nutrition until enteral feeds were established. Plasma malondialdehyde (MDA) and nitrotyrosine were measured by ELISA. Data was analysed by repeated measure ANOVA.

From birth to day 14 total n-6 fatty acids increased while total n-3 fatty acids decreased (p<0.05). Linoleic acid, the precursor of arachadonic acid, increased significantly (p<0.001) while DHA decreased (p=0.01). No significant alteration was observed for EPA. While plasma markers of oxidative stress did not demonstrate a significant relationship with n-6 fatty acids, both MDA and nitrotyrosine were inversely correlated with erythrocyte membrane DHA (p<0.05).

In extremely preterm infants current nutritional strategies fail to meet essential fatty acid requirements with a shift in balance from anti-inflammatory omega-3 fatty acids to pro-inflammatory omega-6 fatty acids. This shift in erythrocyte membrane fatty acid profile is associated with on-going oxidative stress. Despite increasing clinical use of subsequent enteral omega-3 fatty acid supplementation in these high risk infants, the long term consequences of this initial perturbation in essential fatty profile including effects of growth and development, inflammation and immune function remain unknown.

O-2vii-A

Maternal Metabolic Anticipation for Fetal Growth. Alan Jackson. *Invited Speaker, UK.*

O-2vii-B

Maternal Production of Fetal Fuels during Pregnancy: A Comparison of Glucose and Glycine Kinetics between Adolescents and Mature Women. Minerva Thame. *Invited Speaker, Jamaica.*

O-2vii-C

Arginine Flux and NO Synthesis during Pregnancy. A Study of Jamaican, Indian and American Women. Farook Jahoor. *Invited Speaker, USA.*

O-2vii-D

Body Composition Assessment of the Newborn To Evaluate Delivery of Nutrients to the Fetus. Tsinuel Girma. *Invited Speaker, Ethiopia.*

O-3i

The Role of Ouabain/Na,K-ATPase Signaling during Organ Development. Anita Aperia. *Invited Speaker, Sweden.*

O-3i-1

Maternal Protein Restriction Affects Kidney Development by Impairing Nephrogenesis Not Ureteric Branching Morphogenesis. Ryan J. Wood-Bradley, Luise A. Cullen-McEwen, Luke G. Eipper, Debbie Arena, Sarah L. Henry, John F. Bertram, James C. Armitage. *Anatomy and Developmental Biology, Monash University, Victoria, Australia.*

There is a well-established link between maternal protein restriction and low nephron number in adult offspring but little understanding of how kidney development proceeds in the face of protein restriction. Kidney development involves ureteric branching morphogenesis and development of nephrons at the tips of these branches. Thus, reduced nephron endowment could result from impaired branching morphogenesis and/or nephrogenesis.

Here we aim to determine whether nephrogenesis or branching morphogenesis is most impacted by maternal protein restriction.

Virgin Sprague Dawley rats were fed a control normal protein (NP, 20% protein) or low protein (LP, 9% protein) diet ad libitum for three weeks prior to mating, throughout pregnancy and lactation. The LP diet has previously been shown to result in a nephron deficit of 30% in male and female offspring (Hoppe *et al.* Am J Physiol Regul Integr Comp Physiol 292:R462-469, 2007). At embryonic day 14.25 (E14.25) kidneys were cultured for two days, after which the ureteric tree was visualized by wholemount immunofluorescent staining for cytokeratin, and branch points and tips were counted. At E17.5, a combined histochemistry/stereology method (Cullen-McEwen, Armitage *et al.* Am J Physiol Renal Physiol, in press) was used to count developing nephrons. Completion of nephrogenesis was determined histologically by visualizing the nephrogenic zone in early postnatal life (PN5-9).

The number of branch points in the ureteric tree at E14.25 was not altered by maternal protein intake (NP Male (M) 58±4 (mean±SEM), NP Female (F) 52±5, LP M 57±5, LP F 50±3), nor was the number of branch tips (NP M 61±5, NP F 54±5, LP M 58±5, LP F 52±3). However, the number of developing nephrons at E17.5 was significantly reduced in offspring of LP dams (NP M 234.5±7.5, NP F 215.9±7.8, LP M 194.3±15.5, LP F 183.2±15.5, p=0.001). Nephrogenesis ceased between PN7-8 in all offspring.

These data suggest that the reduced nephron number seen in adult offspring exposed to this LP dietary regime during gestation is not caused by reduced branching morphogenesis in utero, but rather reduced nephrogenesis. There was no evidence for premature cessation of nephrogenesis in this model. This study further underlines the importance of maintaining maternal protein intake during mid-gestation, a period of peak nephrogenesis.

O-3i-2

SIRT1 Nutrient Signaling Is Increased during Programmed Fetal Nephrogenesis and Regulates Ureteric Bud Branching. Kevin E. Amaya[1], Sanaz A. Tafti[1], Cynthia C. Nast[2], Mina Desai[1], Michael G. Ross[1], Thomas R. Magee[1]. *[1]Obstetrics and Gynecology, LA BioMed at Harbor UCLA Medical Center, CA, USA; [2]Pathology, Cedars-Sinai Medical Center, CA, USA.*

Intrauterine growth restriction as a result of maternal undernourishment (MUN) results in adult nephropenia and hypertension. Previously, we have shown that MUN leads to reduced glomerular number with dysregulation of genes regulating nephrogenesis during embryogenesis, suggesting that MUN has profound effects on kidney development. To investigate the initial MUN nutrient signaling during nephrogenesis, we examined the expression of nutrient sensor SIRT1 which is also an NAD+ dependent histone deacetylase. To confirm the role of SIRT1 on kidney growth and development, we examined the effects of SIRT1 agonist and antagonist in control explants isolated at E16.

Pregnant rat dams were fed either ad libitum diet (control) or were 50% food restricted (MUN) from E10. Offspring fetal kidneys (n=5 dams for MUN and control) were examined for mRNA and protein expression. At E16, control rat kidneys (n=10 per group) were incubated in DMEM/F12K medium + 10% serum on transwell inserts for three days, with the SIRT1 agonist resveratrol (50 uM) or antagonist EX527 (10 uM). Kidneys were fixed with 4% paraformaldehyde and stained with fluorescein-labeled dolicus bifluoros agglutin, images captured with a confocal microscope and terminal buds quantitated. All values are given as mean ± SEM. Data were considered statistically significant by t-test at $p<0.05$.

Both SIRT1 mRNA (1.6 fold; $p<0.05$) and protein expression (1.7 fold; $p<0.05$) were increased in MUN kidneys compared to controls at E20. SIRT1 also was increased at an earlier time point (E16; 1.9 fold; $p<0.05$), where under-nutrition first impacts fetal weight. Immunohistochemistry detected SIRT1 expression in MUN and control kidneys, primarily in undifferentiated mesenchyme but also in tubules, ducts, and developing glomeruli. E16 kidneys treated with agonist resveratrol had reduced bud tip number of 23%, $p<0.01$; in contrast, treatment with SIRT1 antagonist EX527 increased bud tip number by 57%, $p<0.01$.

Up-regulation of SIRT1 during early MUN nephrogenesis decreases the number of ureteric bud tips and hence ureteric bud branching rate, thus providing a mechanism for the reduction in nephron number seen during nutrient programming. SIRT1 antagonist treatment during nephrogenesis may be an effective therapy for programmed fetal nephropenia.

O-3i-3

Kidney Development in a Hyperglycemic Environment – The Effect of Fetal Weight. Stacey Hokke[1], James C. Armitage[1], Kieran Short[2], Lynelle Jones[2], Ian Smyth[2], John F. Bertram[1], Luise A. Cullen-McEwen[1]. *[1]Anatomy and Developmental Biology, Monash University, Australia; [2]Biochemistry and Molecular Biology, Monash University, Australia.*

Maternal hyperglycemia results in aberrant fetal kidney development including a nephron deficit, but the precise effects of hyperglycemia on two key processes of kidney development (ureteric branching morphogenesis and nephrogenesis) are unclear. This is in large part due to limitations of existing technologies.

We employ 3D optical projection tomography (OPT) and stereology to provide unbiased quantification of branching morphogenesis and nephrogenesis in offspring of diabetic dams and determine if glycemic control in mid gestation prevents the nephron deficit.

Maternal hyperglycemia (~5mmol/L elevation at embryonic day E12.5) was induced by three daily doses of streptozotocin (STZ; 100, 100, 80µg/g body weight) from E6.5-E8.5 in C57BL/6J mice. A subset of diabetic dams had glucose controlled from E13.5 –E18.5 with insulin (120mU/day by osmotic minipump). Ureteric branching morphogenesis was quantified in 3D by OPT at E14.5. Nephron number was assessed using a recently adapted unbiased stereological method at E18.5 (Cullen-McEwen *et al.* Am J Physiol: Renal Physiol, In Press).

Embryos of diabetic dams were intrauterine growth restricted but not developmentally delayed. At E14.5, embryos of diabetic mothers demonstrated ~50% reduced ureteric tree development ($p<0.005$) compared with controls. At E18.5 embryos of diabetic mothers had ~30% fewer glomeruli than controls ($p<0.0002$). Maternal glucose control did not rescue kidney development. Regression analysis identified that fetal weight was highly predictive of ureteric tree development ($p<0.03$) and nephron number ($p<0.001$). Diabetic status was the greatest predictor of embryo weight ($p<0.01$). Regression analysis of nephron number without fetal weight as an independent variable found that nephron number correlated with maternal glucose area under the curve ($p<0.001$). Moreover, 25% of diabetic offspring demonstrated congenital urinary tract abnormalities, including duplex ureters.

Maternal hyperglycaemia results in fetal urinary tract malformations, impaired ureteric branching morphogenesis and impaired nephrogenesis that are proportional to the degree of fetal growth restriction. Insulin therapy reduces maternal hyperglycaemia but not the nephron deficit when instigated in mid-gestation, highlighting the importance of early glycemic control.

O-3i-4

A Maternal Diet Rich in Fat Programmes Sex-Specific Alterations in Renal and Cardiovascular Function in Rats. Sarah L. Henry[1], Ryan J. Wood-Bradley[1], Roger G. Evans[2], Luise A. Cullen-McEwen[1], John F. Bertram[1], James C. Armitage[1]. *[1]Anatomy and Developmental Biology, Monash University, Victoria, Australia; [2]Department of Physiology, Monash University, Victoria, Australia.*

Kidney function in adulthood can be programmed by a range of perturbations to the fetal environment. However, it is not known if renal dysfunction is programmed by excessive maternal exposure to fat during pregnancy. To answer this question we determined renal and cardiovascular function in one year old male and female offspring of fat fed rats.

Female Sprague-Dawley rats (n=5-8) were fed either a control (C) (7% canola oil) or high fat (HF) (3% canola oil and 20% lard) diet for three weeks prior to mating, and during pregnancy and lactation. From weaning, offspring

were chow fed ad libitum. At one year of age glomerular filtration rate (GFR) and effective renal blood flow (eRBF) were estimated in anaesthetized rats by measuring clearance of [^{3}H]-inulin and [14C]-para-aminohippurate. Mean arterial pressure (MAP) and heart rate were determined by radiotelemetry in conscious, freely moving animals. Data were analysed by two way ANOVA, weighted for litter with maternal diet and offspring sex as main factors and Fisher's post hoc tests.

The impact of maternal diet on renal function at one year of age was sex-dependent ($P<0.05$). Male HF offspring (OHF) had greater eRBF (4.95 ± 0.44ml/min/gkw) than did control offspring (OC) (2.88 ± 0.67 ml/min/gkw, $p<0.05$). In contrast, eRBF in OHF females (4.6 ± 1.5 ml/min/gkw) was significantly less than in OC (7.7 ± 1.9ml/min/gkw, $p<0.05$). Maternal diet had a significant sex-dependent effect on filtration fraction (FF) in OHF ($P<0.05$). Male OHF had reduced FF (0.22 ± 0.02) than OC (0.272 ± 0.03), whereas females had significantly greater FF (0.32 ± 0.02) than OC (0.25 ± 0.02). There was no effect of maternal diet on GFR ($P=0.10$). MAP was significantly elevated in OHF compared with OC (male OHF; 98.8 ± 3.6 vs. OC 90.7 ± 2.7 mmHg, $p<0.04$; female OHF 101.9 ± 3.3 vs. OC 90.1 ± 3.3 mmHg, $p<0.05$).

Exposure to a high fat diet during fetal and early postnatal life programmes reduced eRBF, hyperfiltration and elevated MAP in females. Interestingly, male OHF have greater eRBF and increased blood pressure arguing against the hypertension being primarily of renal origin, perhaps implicating neurogenic origins. At present it is not known if the hyperemia in males is advantageous or disadvantageous for longer-term cardiovascular and renal health.

O-3i-5

Maternal Malnutrition Upregulates Expression and Alters Splicing of Angiotensin II Receptor Gene, AT1R, in Prenatal and Neonatal Kidneys. Oleg Denisenko[1], Baoyu Lin[2], Susan Bagby[2,3]. *[1]Medicine, University of Washington, WA, USA; [2]Medicine, OHSU, OR, USA; [3]Portland VA Medical Center, OR, USA.*

Malnutrition during pregnancy causes intrauterine growth restriction (IUGR) and long-term changes in the offspring. The relatively uniform response in offspring - increased risk of developing chronic diseases later in life – despite diverse exposures during pregnancy suggests that common mechanisms may be operative. We recently reported that transcription rates were globally decreased in kidneys from near-term and neonatal LP offspring during MPR. It is well established that inclusion/exclusion of alternative exons is sensitive to gene transcription rates. We hypothesized that global downregulation of transcription during MPR can modify patterns of alternatively spliced transcripts produced from genes related to chronic diseases, thus causing qualitative/quantitative changes in corresponding proteins.

To explore molecular mechanisms by which the intrauterine environment conveys fetal programming, we have used a microswine model of perinatal maternal protein restriction (MPR) where pregnant sows were exposed to low protein (LP) vs normal (NP) diet from .75 Gestation through two wks lactation. Based on prior findings of renal and mesenteric vascular hyperreactivity to AngII in both neonatal and juvenile low-protein offspring, we here examine MPR-induced changes in alternative splicing of the renal cortical Angiotensin Receptor Type 1 (AT1R).

We found that inclusion of exon 2 in AT1R mRNA is significantly reduced in kidneys of LP prenatal and neonatal offspring compared to normal diet controls. Exon 2 encodes a fragment of 5' UTR that contains a small upstream open reading frame, uORF. uORFs are known to slow translation; therefore, AT1R mRNA lacking exon 2 is expected to be translated at higher rates and produce more AT1R protein. In support, we found increased AT1R protein as estimated by autoradiographic analysis of Ang II binding in near-term and neonatal LP vs NP kidneys.

Findings are compatible with the view that, during the period of dietary restriction, MPR induces changes in alternative splicing of AT1R in offspring kidney, potentially through global downregulation of transcription. This may explain increased AT1R protein and altered AngII responses in LP.

O-3i-6

Effects of Uterine Space Restriction (USR) on Ovine Fetal Renal Iron, Nephrogenesis and eNOS. Mary Y. Sun, Katie Meyer, Jill M. Koch, Ronald R. Magness, Pamela J. Kling. *Ob/Gyn, Pediatrics, Nutritional Sciences, University of Wisconsin, Madison, USA.*

Intrauterine growth restriction (IUGR) deriving from many etiologies disrupt iron (Fe) transport and impair renal function in rodent models; Fe status has not been studied in IUGR sheep. Transferrin receptor (TfR) is the major placental cell-surface protein transporting Fe. TfR expression is controlled by iron regulatory proteins (IRP) modulated by nitric oxide (NO) via endothelial nitric oxide synthase (eNOS). We hypothesized that kidneys harvested from a USR model of IUGR will exhibit impaired development, in association with negative Fe status and reduced eNOS expression.

We used an ovine IUGR model of USR (Meyer et. al, BOR, 2010) via single unilateral uterine horn ligation before breeding for 1-3 fetuses. These were compared to sheep fetuses of nonspace restricted (NSR) controls at gestation day (GD) 120 and GD130 (term=147). Blood total iron binding capacity (TIBC), plasma transferrin (Tf), Tf saturation (ferrozine method), and fetal kidney non-heme Fe were quantified. Immunoblotting was performed for TfR and eNOS expression. Cessation of nephrogenesis was determined by identifying an active or inactive nephrogenic zone.

Compared to NSR, USR fetal TIBC,Tf concentration were increased, and Tf saturation decreased at GD130, but not at GD120. Total fetal kidney Fe content (μg) was similar in NSR and USR at GD120 and 130. However when expressed proportionately, μg Fe/kg fetal wt fell from GD120 to 130. Because fetuses were smaller in USR, proportionational Fe levels were greater in USR vs NSR at GD130. There was no difference in renal TfR expression between groups, although renal eNOS expression trended higher in GD130 USR vs both GD120 USR and GD130 NSR. In contrast to placenta, there was no correlation between eNOS and TfR in fetal kidneys. The nephrogenic zone had matured at GD120 in NSR, but matured later in USR.

Thus, contrary to our hypothesis, the IUGR fetuses adapted to USR by delivering more Fe via the blood and accretion by the kidney. Unlike the placenta, renal TfR and eNOS were not associated. Of note, the non-heme Fe assay does not measure red cell Fe, so relatively greater renal Fe is not derived from eNOS-mediated renal vascular dilation. The relatively greater renal Fe may be due in part to the tight regulation of fetal Fe homeostasis during the delay in active nephrogenesis and Fe needs for ongoing cell proliferation during nephrogenesis. NIH HL49210, HD38843, HL87144

O-3ii

Social Determinants of Poor Growth in Early Childhood: Evidence from Birth Cohorts in Low- and Middle-Income Countries. Linda Richter. *Invited Speaker, S. Africa.*

O-3ii-1

Today's Health Is a Function of Yesterday's. Susan Morton[1], Gail Pacheco[2], Mary Hedges[3], Chris Schilling[4], Jatender Mohal[1], Merly Mathew[1]. *[1]School of Population Health, University of Auckland, Auckland, New Zealand; [2]Economics, Auckland University of Technology, Auckland, New Zealand; [3]Economics, University of Auckland, Auckland, New Zealand; [4]New Zealand Institute of Economic Research, Wellington, New Zealand.*

The International Healthy Start to Life Project and Growing Up in New Zealand, a longitudinal study, aim to push the frontiers of life course, epidemiological research by estimating the life course economic costs associated with a less than optimal start to life. We will demonstrate the timeliness and cost-effectiveness of early life interventions for the continuing health of populations both globally and in New Zealand.

Adapting components of previously validated models, Alderman and Behrman's World Bank model and Heckman's human capital formation model, as a baseline, we applied a determinants of change approach to different outcomes across the life course. We acquired longitudinal data sets from Singapore, Aberdeen, Jamaica, Pakistan and Brazil. Initial analysis begins with utilizing data from the SCORM cohort (Singapore Cohort Study of Risk factors for Myopia) collected from Singaporean schoolchildren

(n=662) at birth and at age 11. A series of three ordinal logistic regression and multinomial logit analyses were applied to assess determinants of childhood IQ and changes in IQ.
OLS regression analysis was run with IQ as the outcome and a range of child, household, and school characteristics as independent variables. Mother's education (proxy for cognition at birth) and school characteristics were found to be significant. IQ groups were then divided into five categories and a logistic regression was applied. Mother's education and quality of the school were again significant. The final model analyzed movement across IQ groups from birth to age 11 that were greater or lower than average movements. Father's level of education and school were found to be highly significant for above average movements and in reducing the probability of a below average movement. Other possible influences, such as birth weight, mother's age at birth, and being breast-fed, were insignificant, but had the expected sign on their coefficients, in line with previous research on this front. Results are for use within a micro-simulation model to determine economic gains across the life course.
Estimates derived from these models will serve as key inputs for economic policymakers when determining the cost-effectiveness of interventions from birth to adulthood.

O-3ii-2

Dietary Quality of Young Women: Choice and Quality of Healthy Foods Differ According to Neighbourhood Deprivation. Christina Black[1,2], Georgia Ntani[2], Ross Kenny[2], Tannaze Tinati[1,2], Cyrus Cooper[2], Graham Moon[3], Janis Baird[2]. *[1]NIHR Biomedical Research Unit Nutrition, Diet and Lifestyle, Southampton University Hospitals Trust, United Kingdom; [2]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom; [3]School of Geography, University of Southampton, United Kingdom.*
Disadvantaged women have poorer quality of diet than more advantaged women. The influence of the physical food environment on quality of diet is poorly understood. Research suggests little difference in availability and price of grocery products in poorer and richer neighbourhoods. Few studies have considered differences in variety and quality. We explored the association between dietary quality and neighbourhood deprivation in women in Southampton, UK, and assessed availability, price and variety of products, and fruit and vegetable quality in grocery stores in the city.
We conducted a cross sectional survey of 1022 women between January and July 2009. Women completed a 20-item food frequency questionnaire, used to derive a dietary quality score, and provided their home postcode. A survey of all grocery stores in Southampton assessed availability, cheapest price, variety and quality of 12 food items indicative of healthy and unhealthy dietary patterns. Home and store postcodes were used to determine level of neighbourhood deprivation. We ranked neighbourhoods into fifths according to the national deprivation index in England.
Nearly half the 1022 women (44.8%) lived in the two most deprived neighbourhoods. Neighbourhood deprivation was a significant predictor of quality of diet such that each level increase in neighbourhood affluence corresponded to a 0.21 standard deviation increase in diet score ($p<0.001$, 95% CI 0.16, 0.25). 195 grocery stores were surveyed, 37 in the most and 13 in the least deprived neighbourhoods. There was no difference in availability or cheapest price between neighbourhoods. There was however a significant difference in variety of healthy products (RR=1.17, 95% CI:1.07, 1.27) and quality of fruit and vegetables (OR=0.58, 95% CI: 0.43, 0.80).
Women with the poorest quality diets lived in most deprived neighbourhoods of Southampton. Grocery stores in richer neighbourhoods offered significantly more choice and better quality of healthy foods than stores in poorest neighbourhoods. Public health interventions to enhance choice and quality of healthy foods in poorer neighbourhood stores may reduce dietary inequalities.

O-3ii-3

Exploring the Potential of School Based Adolescent Intervention To Enable Translation of DOHaD Concepts into Community Understanding. Jacquie L. Bay[1,2], Helen A. Mora[1,2], Deborah M. Sloboda[1,2], Mark H. Vickers[1,2], Shawn Cooper[1,2], Peter D. Gluckman[1,2]. *[1]Liggins Instutute, University of Auckland, New Zealand; [2]National Research Centre for Growth and Development, New Zealand.*
A life-course approach to reduction of risk of non-communicable diseases (NCD) suggests that early life interventions may be more effective than traditional lifestyle modifications in middle-age[1]. Translation of DOHaD concepts creates the prospect of response at an individual, family or community level, enabling the use of knowledge in decision making before pregnancy that may reduce NCD risk. Evidence suggests that women do not make sustained dietary change before or during pregnancy[2], making adolescence one appropriate place in the life-course for intervention. We have engaged adolescents in a school based translational intervention, and evaluated the potential of this to enable development of understanding of DOHaD concepts that could support behavior change and reduce NCD risk in the next generation.
[1]Godfrey, K.M, *et al.* Developmental origins of metabolic disease: life course and intergenerational perspectives. *Trends in Endocrinology and Metabolism* 21, 4: 199 – 205, 2010
[2] S.R Crozier *et al.* Women's dietary patterns change little from before to during pregnancy. *J. Nutrition* 139: 1956-1963, 2009
Modules of course work relating to nutrition and development, and presenting examples of DOHaD evidence were written for 11-14 year olds (grades 7-10) and trialed in 10 schools. Professional development was provided for teachers. Pre and post intervention questionnaires and post intervention interviews were used to evaluate the intervention.
Recognition of a strong link between the diet of the mother during pregnancy and the health of the fetus as an adult moved from 14% to 32% as a result of the intervention. Evidence 6-12 weeks post intervention suggests that it has facilitated discussion of diet, lifestyle and DOHaD concepts in some families.
Initial post intervention analysis suggests that the intervention has been effective in changing understanding of DOHaD concepts and in some cases led to behavior change. However, the sustainability of these changes remains to be determined through ongoing evaluation of attitudes and behavior within this cohort.

O-3ii-4

Developmental Origins of Health Disparities in the U.S.: A Clinical Case Study. Melanie Thomas, Anna Spielvogel, Frances Cohen, Susan Fisher-Owens, Betsy Wolfe, Martha Shumway. *University of California, San Francisco, USA.*
The U.S. has a high incidence of low birth weight (LBW) outcomes in comparison with other developed countries and there are well-documented racial/ethnic disparities. The goal of this study was to examine the influence of appointment attendance and psychiatric and medical diagnoses on LBW disparities within a high risk clinic population.
Data were retrospectively obtained from clinical databases on all women scheduled for appointments in the San Francisco General Hospital (SFGH) High Risk Obstetrics (OB) clinic during two different three month periods. General linear model procedures were used to examine attendance, psychiatric and medical diagnoses, and birth outcomes by race/ethnicity.
Study 1: During the three month study period, 327 women had appointments scheduled at the SFGH high risk OB clinic. For OB services, Hispanic (77%) and Asian (75%) women attended more scheduled appointments than African American (52%) and White (54%) women ($F= 8.14$, $p<.0001$). For psychiatry services, differences were not significant, although Hispanic (43%) and White (43%) women attended more scheduled appointments than African American (33%) and Asian (31%) women.
Study 2: In the second study period, 217 women had available data on medical and psychiatric diagnoses and birth outcomes. Race/ethnicity was associated with diagnoses and LBW outcomes. The prevalence of medical and psychiatric diagnoses in combination was higher among African American women (39%) than other racial/ethnic groups (Hispanic 17%, Asian 15% and White 15%, $X2(9)=26.8$, $p<.01$). Twenty-two percent of

African American mothers had LBW infants, 9.2 times more often than white women (X2(1)=4.2, p=.04). Diagnosis of medical or psychiatric illness independently and concurrently did not correlate with LBW outcomes.
The roots of racial disparities in U.S. birth outcomes are complex. Given the various consequences of LBW over the lifecourse, understanding and eliminating these disparities is a critical public health issue. In our clinical sample, all women have the same access to healthcare and are from similarly low SES. However, African American women remain more likely to have LBW infants. LBW outcome does not correlate with any diagnoses, but African American women were more likely to have combined medical and psychiatric diagnoses. Next steps include integrating appointment attendance and outcome data.

O-3ii-5

Adolescence Pregnancy: Intergerational Déjà-Vu? Alexandre A. Ferraro[1], Carlos A. Faria[2], Viviane C. Cardoso[3], Carlos Grandi[4], Aline P. Barbosa[3], Ricardo C. Cavalli[3], Antonio A.M. da Silva[5], Heloisa Bettiol[3], Marco C. Barbieri[3]. *[1]Faculdade de Medicina da Universidade de SP - Campus Capital, Brazil; [2]Universidade Federal Fluminense, Brazil; [3]Faculdade de Medicina da Universidade de SP - Ribeirão Preto, Brazil; [4]Maternidade Sardá, Argentina; [5]Universidade Federal do Maranhão, Brazil.*
There is plenty of evidence which suggests that pregnancy in adolescence is related to social risk. The aim of this study is to analyse the effect of pregnancy history in adolescence in one generation on its reincidence in the next generation in a cohort of women born in Brazil and to know whether this relationship keeps present after adjustments for potention biological and socioeconomic condounders.
A prospective cohort of all living born of the city of Ribeirao Preto, Brazil, was assessed at birth (1978/79) and adulthood (2002/04). Data on socioeconomic position at both moments, as well as on biological factors were present for 1059 pairs of mother-daughter, corresponding 32% of the eligible subjects. Poisson regression was performed to estimate the crude and adjusted risk rates.
The incidence rate of adolescence pregnancy in the first generation was 31.4% and in the second 17.1%. All socioeconomic markers in both moments were associated with the reincidence, as well as age of menarch and ethnicity. The effect of having a mother who was a pregnant adolescent on having a adolescence pregnancy was 1.66 after adjusting for socioeconomic postion at birth and adulthood, age in which started working,ethnicity and age of menarch. When the education (in the 1st and 2nd generation) was included in the model, the effect turned to RR= 1.26 and the p=0,072.
Pregnancy in adolescence in the first generation is a predictor of its reincidence in the second generation, even after adjusting for specific biological and socioeconomic factors.

O-3ii-6

The Use of a Geographic Information System to Determine Environmental Influence in Low Birth Weight in the Western Region of São Paulo: The Butantã Cohort. Alexandra Brentani, Filumena Maria S. Gomes, Maria Helena Valente, Izadora S. Santos, João Henrique de Sá, Marco Antonio Gutierrez, Ana Maria U. Escobar, Sandra Josefina F.E. Grisi. *Pediatrics, Faculdade de Medicina da Universidade de São Paulo, SP, Brazil.*
Introduction: Interventions at the beginning of a disease or, better yet, even before its onset, when the chances of cure are greater and the costs are lower, seem to be a more effective approach to chronic problems. Several studies found associations between insults sustained during early developmental stages and greater risk of chronic diseases and mental disorders, expressed by means of epigenetic mechanisms or the structuring of the subjective self by means of affective experiences in the beginning of life. Study results may provide subsidies for significant changes in prevention, health promotion in different stages of life and improvement of the cost-effectiveness of public policy actions.
Objectives: to study possible correlations between low birth weight and environmental conditions at birth.
Based on data from 137 medical records available from the Butantã cohort we formed our study group, comprised of patients who had low birth weight (i.e., who weighed less than 2,500 g, at birth (Definition of the World Health Organization)). The Butantã cohort is located in the western region of São Paulo City and is part of a research project of the Pediatrics Department. Besides birth weight, address and information on living conditions (type of residence building, waste disposal, water source, and distance from the health unit) were also collected. A geographical information system enabled the development of the analysis, correlating birth weight to the outcomes of interest (local and living condition). Frequency of occurrence of outcomes in the sample was than calculated.
4.2% (n=6) children lived inside the area of the health unit coverage (less than 3 km); 60% (n=85) of children lived as far as 5 km from the unit and 35,5% (n=32) lived in the coverage area of another health unit. 45.2% (n=62) lived in slums, 47% lived in a single room house and only 8% of the children lived in houses bigger or equal two rooms.
There is a concentration of low birth weight children living in poorest conditions and far from the health units, which may have contributed to a poorer pre natal follow-up.

O-3iii-1

Whole Transcriptome Shotgun Sequencing (RNA-SEQ) in a Murine Model of Antenatal Glucocorticoid-Rescued Pulmonary Immaturity Reveals Essential Roles for Surfactant Protein B (SPB) and Corticotropin Releasing Hormone (CRH). Kjersti Aagaard[1], Alan Harris[1], Milenka Cueva[1], Cynthia Shope[1], Rui Chen[1], Bryce Daines[1], Sonia Klinger[2], Sylvain Meloche[2]. *[1]Obstetrics&Gynecology, Baylor College of Medicine, USA; [2]Institute de Recherche en Immunologie et Cancrologie, Universite de Montreal, Canada.*
Despite over three decades of use, the mechanisms by which in utero glucocorticoids promote fetal lung maturity are not well understood. We reasoned that whole transcriptome shotgun sequencing (NexGen RNA-Seq) in biologically relevant models would reveal potential gene signature pathways by which glucocorticoids antenatally function.
We previously reported that in our Erk3-/- MAP kinase family knockout (ko) mouse model, 0.4mg/kg dexamethasone or saline administered at E16.5 and E17.5 rescued pulmonary immaturity (decreased sacculation and increased type II pneumocyte glycogen; PNAS 106:16710, 2009). To significantly expand this work, high integrity (>8.5, Agilent bioanalyzer) coding 3' polyadenylated mRNA was extracted from flash-frozen lung E18.5, and sequencing libraries were generated from random-hexamer synthesized cDNA. <400bp fragmented cDNAs served as sequencing template (Illumina GAII). Transcriptome alignment was against murine reference reads(NCBI), and CuffDiff FPKM algorithms inferred expression level (means of read normalization). IPA analysis deciphered novel pathways, and significant estimates were validated with gene-specific QPCR.
IUGR Erk3-/- neonatal mice died within 24 hours from acute respiratory failure; in utero dexamethasone rescued histologic lung maturation and differentiation of type II cells but not neonatal lethality. Employing RNA-Seq, 114 million sequence reads were generated. Following in silico subtractive hybridization and extensive data mining, two significant glucocorticoid-induced signature pathways in the neonatal lung emerged: corticotropin (CRH, CRH receptor 2, urocortin) and surfactants (SPB but not SPA). In situ hybridization and immunohistochemistry on biologic replicates validated these observations.
Antenatal glucocorticoids decrease pulmonary CRH in an Erk3-independent pathway (i.e., histologic type II maturation), and increase Erk3-dependent SPB (i.e., functional pulmonary maturation). Our unbiased RNA shotgun sequencing of fetal signature pathways reveals a previously undescribed role for Erk3, CRH and SPB in fetal pulmonary maturation.

O-3iii-2

Intra Uterine Growth Retardation Induced Adult Male Sub-Fertility Is Corrected by Placental Gene Therapy with Insulin like Growth Factor-1. Suzi Demirbag, Mounira Habli, Ursula Harkness, Helen Jones, Foong Yen Lim, Sundeep Keswani, Timothy Crombleholme. *Cincinnati Childrens Hospital, OH, USA.*
We have previously shown that placental gene transfer of Insulin-like Growth Factor-1 (IGF-1) corrects growth in models of IUGR. IGF-1 has a major influence on fetal and postnatal growth. We hypothesized that IUGR induced adult male sub-fertility can also be corrected by placental gene

therapy. To test this hypothesis we examined the effect of recombinant adenoviral-mediated placental gene transfer of IGF-1 (Ad-IGF-1) on postnatal testes histology.
Time mated Sprague-Dawley rats were treated on day 18 of a 23-day gestation; Control (n=4), Uterine Artery Ligation (UAL, n=3), and UAL+ Ad-IGF-1 (1x108 & 1x109 PFU, n=9) injected on day 18. Adult male rats were sacrificed at 36 weeks and testicles were harvested. Morphometric analysis was performed was using Seminiferous Tubule Area (STA), Germinal Layer Area (GLA),as well as quantification of Sertoli cell, Spermatogonia, and Spermatocyte counts per tubule. Statistical analysis was performed using ANOVA.
UAL resulted in a significant reduction in STA compare to control (0.035±.01 vs,0.44±.08mm2,p=0.001). UAL+Ad-IGF-1 resulted in a statistically significant increase in STA compared to UAL alone (0.32±.09 vs 0.035±.01 mm2,p=0.001). UAL resulted in a significant lower GLA compared to control rats (0.01±.001 vs 0.3±.01mm2, p=0.001) and significantly higher in UAL+AdIGF-1compare to UAL (0.01±.001vs0.2±.007 mm2, p=0.001). Sertoli cell counts were significantly lower in UAL compare to control (11.11± 3 vs 33.77 ±1.8, p=0.007) and significantly higher in UAL+AdIGF-1compare to UAL (26.67±1.87vs11.11±3, p=0.02). Spermatocyte counts were significantly lower in UAL compare to control (103.77±9.10vs229.33±21.71, p=0.01) and significantly higher in UAL+Ad-IGF-1 compared to UAL (229 .33±21.71vs103.77±9.10,p=0.01). Spermatogonia counts were significantly lower in UAL compare to Control (39.66±11.54 vs 66.66±2.99, p=0.03). UAL mediated IUGR results in sub-fertility in adult males.
Placental gene transfer of IGF-1 partially corrects adult male sub-fertility. These results provide proof of concept that placental gene therapy may be an effective strategy for correction of adult disease related to IUGR such as male sub-fertility. These results may become implemented for other adult diseases associated with IUGR.

O-3iii-3

Exercise as an Intervention in Reducing the Impact of Maternal Obesity in Female Rat Offspring. Hasnah Bahari, Vanni Caruso, Margaret Joan Morris. *Pharmacology, University of New South Wales, New South Wales, Australia.*
Intrauterine adaptations due to maternal obesity lead to changes in metabolism and brain appetite regulators in the offspring at adulthood (Rajia *et al*. 2010). Exercise is important in preventing cardiovascular and metabolic disease, and we previously showed it reduced adiposity in offspring of obese mothers. However the question of whether exercise in adulthood might be beneficial in offspring exposed to maternal obesity has not been addressed previously.The aim is to examine the effects of short term exercise implemented late in life, on adiposity and hormone profile in the female offspring of obese rat mothers.
Adult Sprague Dawley rats were fed either normal chow or high fat chow diet (HFD) ad libitum for five weeks to yield chow and HFD mothers. Next, they were mated with chow fed Sprague Dawley rats. Dams consumed same diet throughout gestation and lactation period. At weaning, female rats from each litter were separated into two diet groups chow (C) or HFD (F) and after seven weeks on their respective diet, half from each group were exercised (voluntary running wheels) for five weeks while the remainder were sedentary (S).
After seven weeks of sedentary period, postweaning F diet significantly increased BW and RpWAT mass in offspring from both C and F mothers compared to offspring fed C postweaning ($P<0.05$). Exercise for five weeks significantly decreased BW, RpWAT mass (decrease of 17% and 35% respectively), plasma leptin and insulin concentrations in offspring of F mothers consuming F postweaning compared to sedentary group ($P<0.05$). Exercise improved the phenotypic profile caused by maternal obesity and postnatal overnutrition. Some benefit was also observed in offspring of F mothers consuming C. Following five weeks of late onset exercise, offspring from C mothers consuming F had significant reductions in BW and RpWAT mass (reduction of 13% and 46% respectively) compared to the sedentary group ($P<0.05$).
A short period of exercise implemented post puberty had beneficial effects in decreasing BW, adiposity, leptin and insulin in offspring from F mothers, with greater effects in rats fed F at postweaning. Offspring of C mothers who consumed F also showed reduced adiposity post exercise.

Rajia S, Chen H, Morris MJ. Maternal overnutrition impacts offspring adiposity and brain appetite markers-modulation by postweaning diet. J Neuroendocrinology 2010; 22(8):905-914.

O-3iii-4

Leptin and PPARα: Understanding Their Role in Preventing Obesity in the IUGR Rat. Emma Garratt[1], Mark Vickers[2], Peter Gluckman[2], Mark Hanson[1], Graham Burdge[1], Karen Lillycrop[3]. *[1]Human Development and Health, University of Southampton, United Kingdom; [2]Liggins Institute and the National Research Centre for Growth and Development, University of Auckland, New Zealand; [3]Developmental and Cell Biology, University of Southampton, United Kingdom.*
Environmental constraints acting during early development can induce an increased risk of disease in later life. In a rat model of IUGR, adult offspring are obese with associated metabolic disturbances. These are augmented by a HF postnatal diet and prevented by neonatal leptin treatment. To determine the mechanism of leptin reversal, we investigated the effect of neonatal leptin treatment and a postnatal HF diet on the expression of genes involved in fatty acid metabolism and energy balance in the adipose tissue of adult offspring from control and UN dams. In order to investigate the regulation of PPARα by leptin, tissue specific PPARα promoters were identified and characterized.
Dams were fed either ad libitum (AD) or 30% ad libitum (UN) intake throughout pregnancy. Leptin (rec-rat, 2μg/g/day, sc) was administered to female offspring from postnatal days 3-13. Offspring were weaned onto either a control chow or HF diet and killed at 170 days. Expression of PPARα, PPARγ and their target genes AOX, CPT-1 and LPL was measured in adipose tissue by RTPCR. PPARα transcripts were identified by 5'RLM RACE and their promoter activity measured using a reporter gene strategy.
Expression of PPARα, PPARγ and their target genes was increased in all leptin treated offspring fed a HF diet. Alternative PPARα transcripts were identified in adipose tissue (P1) and liver (P2). Leptin had no effect on P1 promoter activity but induced P2 promoter activity via a STAT3/Sp1 dependent mechanism. Neonatal leptin treatment induced transcription of the P2 but not the P1 transcript in adipose tissue of AD and UN offspring. These data suggest that leptin treatment during adipogenesis induces a persistent change in adipose fatty acid metabolism through specific activation of a quiescent PPARα promoter. Thus, the reduced weight gain induced by neonatal leptin treatment in these offspring could be due to the persistent increased expression of PPARα, PPARγ and their target genes inducing altered energy balance within the adipocytes. This suggests that neonatal leptin exposure may have persistent peripheral metabolic effects as well as effects on hypothalamic neurogenesis.
Supported by BHF, HRC New Zealand and NRCGD.

O-3iii-5

Increased Amino Acids Potentiate Glucose Stimulated Insulin Secretion but Do Not Increase Beta Cell Mass in the Ovine Fetus. Monika Gadhia, Anne Maliszewski, Meghan O'Meara, Stephanie Thorn, Jinny Lavezzi, William Hay, Laura Brown, Paul Rozance. *Pediatrics, University of Colorado, CO, USA.*
Growth restricted fetuses have reduced amino acid supply and are also characterized by decreased insulin secretion. It is unknown whether chronically increased fetal amino acid supply would stimulate insulin secretion, even in normally growing fetuses. We hypothesized that chronically increasing the fetal amino acid supply would increase glucose stimulated insulin secretion (GSIS) in the normally grown ovine fetus.
Singleton ovine fetuses at 113-120 days gestation (term=148 days) were given a direct intravenous infusion of a complete amino acid mixture (AA group, n=8) or saline (CON group, n=8) for 10-14 days. The infusion was increased in the AA group to target a 25-50% increase in fetal branch chain amino acids (BCAA). BCAA, glucose, insulin, pH, blood gasses, and hematocrit were measured throughout the infusion. On the final day of infusion, fetal GSIS and arginine stimulated insulin secretion (ASIS) were measured using a square wave hyperglycemic clamp and arginine bolus. Pancreatic insulin content was measured with ELISA and beta cell mass with immunoflourescence staining.

Fetal BCAA concentrations increased 50% in the AA group compared to CON ($p<0.05$). Glucose decreased over time in the AA group (22.7±1.5 baseline vs. 17.9±0.4 mg/dl final day, $p<0.01$) but not in CON. pO_2, O_2 saturation, and O_2 content trended down during the middle of the infusion in the AA group, but this difference was not significant and values returned to baseline. Hematocrit, pH, and insulin concentrations were similar between the groups during the infusion period. During the GSIS study, there was a pronounced increase in early phase insulin concentrations (15 min: 2.06±0.53 AA vs. 0.80±0.20 ng/ml CON, $p<0.01$) and this difference was sustained during the hyperglycemic clamp in the AA group (105 min: 1.38±0.23 AA vs. 0.70±0.14 ng/ml CON, $p<0.001$). ASIS and pancreatic insulin content were similar between groups. At necropsy, fetal carcass weights and crown rump lengths were similar.

Chronically increased amino acid supply to the normally grown ovine fetus in late gestation potentiated insulin secretion in response to glucose. Because GSIS was potentiated, ASIS was not, and pancreatic insulin content and beta cell mass were not different, we speculate that amino acids upregulate glucose metabolism and generation of secondary messengers in the beta-cell.

O-3iii-6

Effects of Maternal Obesity on Offspring Are Exacerbated by Overnutrition in Adulthood and Ameliorated by Early Exercise Intervention. Vanni Caruso, Hasnah Bahari, Margaret J. Morris. *Department of Pharmacology, School of Medical Sciences, University Of New South Wales, NSW, Australia.*

The development of obesity is influenced by the interaction of genetic and environmental factors. The intrauterine environment plays a key role in the development of adult metabolic disease, resetting the expression of genes involved in energy homeostasis. Maternal obesity is increasing in developed countries. Here we aimed to test whether early exercise could have beneficial effects on offspring following maternal obesity. We examined the effects of an early exercise intervention on adiposity and hormone profile of male offspring of obese mothers consuming either chow (C) or a high fat chow diet (HFD).

Adult female Sprague Dawley rats were fed either normal chow or HFD ad libitum for five weeks, then mated with chow fed male Sprague Dawley rats. Dams continued on their assigned diet during lactation. At weaning (week 3) male rats were separated into two diet groups, chow or HFD (F); half were exercised (voluntary running wheels), while the remainder were sedentary. At week 10, exercise wheels were removed and rats were euthanased at 15 weeks for tissue and plasma collection.

Maternal obesity increased pup adiposity at 15 weeks and this was exacerbated by post-natal HFD ($P < 0.05$). Seven weeks of voluntary exercise reduced body weight (BW) at 10 weeks of age in rats consuming chow but not HFD. The reduction of BW and fat mass in this group was maintained even after five weeks without exercise suggesting that early exercise may prevent subsequent weight gain due to later sedentary lifestyle. At 15 weeks, fasting plasma insulin level was significantly reduced by exercise (49%) in offspring of obese mothers consuming HFD, compared to those remaining sedentary and consuming the same diet.

A short period of exercise early in life had long lasting beneficial effects on the BW, adipose mass and hormone profile of male offspring from obese mothers, despite being followed by a period of inactivity.

O-3iii-7

The Effect of Maternal Taurine Supplementation on Timing of Pubertal Onset in Male and Female Offspring Is Directionally Dependent upon Maternal Nutritional Background. Deborah M. Sloboda, Minglan Li, Mark H. Vickers. *Liggins Institute, University of Auckland and the NRCGD, New Zealand.*

We have previously shown that maternal nutritional history influences pubertal onset, obesity and metabolic function. More recently, we set out to examine maternal fructose intake as a model, due its relevance to modern human diets. We have shown that maternal fructose intake altered fetal and placental growth and neonatal hepatic lipid accumulation in a sex-specific manner. In the present study we set out to determine the long-term effects of maternal fructose and high fat intake on offspring pubertal onset and whether maternal taurine supplementation would modify these effects.

Pregnant Wistar rats were randomized to receive control ± taurine (1-2% wt/vol based on fluid intake) (CON; CON-T), fructose ± taurine (Fr; Fr-T), or high fat+fructose ± taurine (HFr; HFr-T) during pregnancy and lactation. Maternal food intake, growth, and offspring food intake and growth were recorded throughout pregnancy, lactation and post-weaning time periods respectively. Offspring were examined for pubertal onset from postnatal day 28 using vaginal opening and balanopreputial separation in females and males respectively.

Maternal intake of both Fr and HFr advanced pubertal onset in male and female offspring ($p<0.05$). Intriguingly, in CON-T, maternal taurine intake alone also advanced pubertal onset in male and female offspring ($p=0.004$). In contrast, maternal taurine supplementation in Fr dams normalized pubertal onset in female offspring back to CON values, and HFr-T had no further effect. In contrast to females, maternal taurine supplementation had no effect on pubertal onset in male offspring beyond that observed with Fr or HFr. Overall, maternal taurine supplementation during pregnancy and lactation in control animals, similarly advanced pubertal onset in male and female offspring ($p<0.01$) as observed with maternal Fr and HFr intake.

These data demonstrate that maternal Fr and HFr intake during pregnancy and lactation have long-term effects on offspring reproductive maturation and that in these groups, taurine reversal is dependent upon maternal nutritional history and offspring sex. Further, it appears that maternal taurine intake alone may also compromise offspring phenotype. Ongoing studies will determine whether the observed sex differences elicit different risk profiles for metabolic and reproductive compromise into adulthood.

O-3iv

Transition, Complexity and Public Health: How Important Is DOHaD to Low-Income Countries? David Osrin. *Invited Speaker, UK.*

O-3iv-1

Slum Redevelopment: Major Constraint for DOHaD Research in Urban Slums of India. Sujay Joshi[1], Ramesh Potdar[1], Meera Gandhi[1], Caroline Fall[2]. *[1]Centre for the Study of Social Change, Mumbai, India; [2]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom.*

A study of out-migration patterns among women registered in an ongoing randomized controlled trial (RCT) in urban slums of Mumbai city in India.

The Mumbai Maternal Nutrition Project (Project 'SARAS') is an ongoing RCT of food-based micronutrient supplementation in slum women before and during pregnancy, with the primary outcome of increased birthweight, and long-term outcomes of improved child growth and development. Micronutrients are delivered in the form of snacks made from green leafy vegetables, milk and fruit to be eaten under observation at least three times a week. Babies born in the study are followed up with anthropometry and neuro-developmental assessment (DASII: Developmental Assessment Scale for Indian Infants). Since the trial began, municipal Slum Redevelopment (SR) schemes, aimed at making Mumbai slum-free, have become a major activity, creating out-migration of families, and thus concern about long-term follow-up of the babies.

Data were collected by trained SARAS health workers using a pretested questionnaire.

552 women have migrated out of the study area (from a total of 6672 registered women). Of these, 65 shifted temporarily (non pregnant=31, pregnant=8, mothers with babies=26) and 487 shifted permanently (non pregnant=369, pregnant=5, mothers with babies=113). Migration was highest during 2007 (n=102), 2008 (n=146) and 2009 (n=126) due to the launch and continuation of the SR scheme. Migration was minimum (n=38) in 2006, the starting year of the trial, when there were no SR schemes, and in 2010 (n=75), when migrated families came back to the study area. Fortunately 42% shifted to Mumbai suburbs (1-30 km), where continued follow-up is feasible, while 45% shifted far away (50-500 km) and 13% could not be traced at all. Thus permanent attrition among women (non-pregnant and pregnant currently registered in trial) was 12.10% (n=374/3089). Amongst 113 mothers with babies, 53% shifted to Mumbai suburbs (1-30 km) while 21% shifted far away (50-500 km) and 26% could not be traced.

Though randomized control trials are seen as the gold standard in evaluative research, they are still vulnerable to biases like attrition, which can

undermine their internal validity. Our findings indicate a need to develop special strategies to minimize attrition and increase engagement of SARAS families for follow up of the babies.

O-3iv-2

Effects of Prenatal Food and Multiple Micronutrient Supplementation on Mortality and Risk Indicators of Metabolic Syndrome at 5 Years of Age. The MINIMat Trial in Rural Bangladesh. Lars-Åke Persson[1], Shams Arifeen[2], Ashraf I. Khan[2], Iqbal Kabir[2], Emma Lindström[1], Eva-Charlotte Ekström[1]. *[1]International Maternal and Child Health, Women's and Children's Health, Uppsala University, Uppsala, Sweden; [2]Public Health and Clinical Sciences Divisions, ICDDR,B, Dhaka, Bangladesh.*

This trial evaluates short- and long-term effects of combined food- and multiple micronutrient supplementation to undernourished women in Bangladesh.

In the MINIMat prenatal nutrition trial in Bangladesh (ISRCTN16581394) 4436 pregnant women were randomized into six equally sized groups; a double-masked supplementation with either 30 mg Fe and 400 µg folic acid, or 60 mg Fe and 400 µg folic acid, or a multiple micronutrient supplement containing a daily allowance of 15 micronutrients including 30 mg Fe and folic acid starting at around 14 wk, was combined with a randomized early invitation (from around 9 wk) or a usual invitation to start (from around 16 wk) food supplementation (600 Kcal six days per week).

Among the 3625 live births there was 66% reduction of under-five mortality by an early timing of prenatal food supplementation if combined with multiple micronutrients. An early initiation (vs. usual timing) of prenatal food supplementation resulted in less stunting 0-5 yrs and a more favourable lipid profile at 5 yrs, while prenatal multiple micronutrients (vs. iron-folate) was linked to more stunting, lower IGF1 and insulin levels.

These combined prenatal nutrition interventions resulted in major improvement in child survival combined with nutrition and metabolic effects at 5 yrs. This underlines the need to analyse multiple and long-term outcomes of prenatal nutrition interventions and to identify favourable as well as unfavourable effects.

O-3iv-3

Insulin Reverses Gestational Diabetes-Increased L-Arginine Transport Via A_{2A} Adenosine Receptors Activation in Human Umbilical Vein Endothelium. Enrique Guzmán-Gutiérrez, Carlos Puebla, Carlos Salomón, Francisco Westermeier, Andrea Leiva, Paola Casanello, Luis Sobrevia. *Cellular and Molecular Physiology Laboratory (CMPL) & Perinatology Research Laboratory (PRL), Division of Obstetrics and Gynecology, Pontificia Universidad Católica de Chile, Región Metropolitana, Chile.*

In HUVEC from pregnancies with Gestational Diabetes, lower expression and activity of the nucleoside transporter-1 (hENT1) has been described. This is associated with extracellular Adenosine accumulation, A_{2A} receptor activation and higher L-arginine transport in this placental vascular cell type. GD effect on hENT1 activity and extracellular adenosine concentration is blocked by insulin. We evaluated whether insulin reverses GD-activated L-arginine transport involving adenosine receptors.

L-Arginine uptake (300 µM, 3 µCi/ml, 37°C, 1 min) was measured in HUVEC from normal (N) or GD (GD) pregnancies. Cells were preincubated (8 h) with insulin (1 nM) in absence or presence of adenosine receptors agonist 5'-*N*-ethyl-carboxamidoadenosine (NECA, 30 nM), A_{2A} adenosine receptor antagonist ZM-241385 (10 nM), or adenosine transport inhibitor nitrobenzylthioinosine (NBTI, 10 µM). Adenosine in the culture medium was measured by hplc.

We confirmed that GD is associated with increased L-arginine uptake, and that insulin has a similar effect in N cells. Insulin reduced GD-increased L-arginine uptake (68 ± 3%), an effect blocked by NBTI, while insulin increased L-arginine uptake in absence (1.9-fold) or presence (6.7-fold) of NBTI in N cells. In absence of insulin NECA did not alter L-arginine uptake in GD cells; however, NECA blocked insulin-reduced uptake in GD cells. In N cells, NECA increased L-arginine uptake in absence of insulin (1.8-fold), an effect higher in presence of this hormone (4.3-fold). ZM-241385 blocked GD effect, as well as insulin effect in N cells; however, reversed only partially GD effect on transport in the presence of insulin. NECA effects were blocked by ZM-241385. Insulin reduced GD-increased extracellular adenosine in HUVEC, an effect blocked by NBTI. In N cells insulin increased extracellular adenosine.

Insulin reverses GD-associated alterations in L-arginine transport involving A_{2A} adenosine receptors in HUVEC. We suggest that insulin could be acting as a modulatory factor reversing fetal endothelium phenotype associated with insulin resistance to a normal phenotype. Additionally the vascular programming observed in GD could be reverted by insulin.

O-3iv-4

Association between Parental Body Mass Index and Body Mass Index in the Offspring: New Delhi Birth Cohort. Poornima Prabhakaran[1], Dimple Kondal[1,2], H.P.S. Sachdev[3], Santosh Bhargava[4]. *[1]Public Health Foundation of India, New Delhi, India; [2]Centre for Chronic Disease Control, New Delhi, India; [3]Sitaram Bhartia Institute of Science and Research, New Delhi, India; [4]Sunderlal Jain Hospital, New Delhi, India.*

Background and Objectives – Recent research, largely in developed countries, has shown that a graded association exists between parental weight status and adiposity in the offspring. Evidence is inconsistent with respect to the differences, if any, between the degree of association for mother-child (intra-uterine plus genetic and/or shared household) versus father-child (genetic and/or shared household) effects in the assessment of intergenerational influences. In developing countries, this evidence is much more limited. We examined the association of parental BMI on BMI in the offspring and the differences, if any, between mother –child and father-child associations in the New Delhi Birth Cohort in India.

Methods – The study population consisted of male and female members (F1) of the New Delhi Birth Cohort (n= 700, mean age (SD) 24.9(4.4)) and their offspring F2 (n=1210, mean age (SD) =7.7(3.9). Prospectively collected anthropometric data of height and weight was available for all cohort subjects and Body mass Index was calculated as weight (Kg)/Ht (m^2). We generated internal sex-specific SD scores for BMI in this population. The effects of parental BMI on offspring BMI were assessed separately for male and female cohort members using regression analyses and was adjusted for familial clustering.

Results – There is a significant association between overall parental BMI and offspring BMI after adjusting for offspring age and familial clustering[β(SE)=0.2978(0.0281), 95% CI= (0.2425, 0.3529)].The strength of association between the female cohort –offspring BMI was more [β(SE)=0.3647(0.0458), 95% CI= (0.2744, 0.4549)]as compared to the male cohort-offspring BMI [β(SE)=0.2498(0.0344), 95% CI= (0.1821, 0.3176)].

Conclusions – The possibly stronger associations that mothers have with offspring BMI as compared to fathers with offspring BMI point to intergenerational mechanisms, possibly through intra-uterine programming influences that have an additive effect over and above genetic and shared household lifestyle and behavioural effects. This is in contrast to the findings from many developed countries where there are there are no differences between maternal –offspring and paternal –offspring associations for BMI.

O-3iv-5

Birth Size, Serum Cortisol and Cardiometabolic Risk Markers in Indian Children. G.V. Krishnaveni[1], S.R. Veena[1], J.C. Hill[2], C.H.D. Fall[2]. *[1]Epidemiology Research Unit, Holdsworth Memorial Hospital, Mysore, India; [2]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom.*

Exaggerated HPA axis activity may be an important mechanism linking the muscle-thin but adipose (thin-fat) Indian phenotype with the development of adult type 2 diabetes and cardiovascular disease. We hypothesized that smaller size but greater adiposity at birth is associated with higher plasma cortisol concentrations in 9.5 year old Indian children, and that higher cortisol concentrations are associated with elevated cardiometabolic risk markers.

Plasma cortisol and corticosteroid binding globulin (CBG) concentrations were measured in children from an ongoing birth cohort at 9.5 years of age (N Max=532). They had detailed anthropometry at birth, and anthropometry, plasma glucose, insulin and lipid concentrations, and blood pressure (BP) measured at 9.5 years of age. Insulin resistance was calculated (IR-HOMA).

Median (IQR) plasma cortisol concentration was 256.3 nmol/l (191.6, 359.6) and CBG concentration was 54.3 µg/ml (44.5, 65.8). An SD increase in current weight was associated with a 0.14 SD decrease in cortisol (95% CI: -0.22,-0.06; p<0.001 adjusted for age and sex) and a 0.10 SD decrease in CBG concentrations (95% CI: -0.18,-0.02; p=0.02). Higher plasma cortisol concentrations were associated with higher fasting glucose (β=0.25 SD, 95% CI: 0.17,0.34; p<0.001 adjusted for age and sex, socio-economic status and current weight), 30-minute glucose (β=0.13 SD, 95% CI: 0.04,0.21; p=0.004) and triglyceride concentrations (β=0.09 SD, 95% CI: 0.004,0.17; p=0.04), and systolic BP (β=0.13 SD, 95% CI: 0.06,0.21; p=0.001). There was no association with insulin concentrations and IR-HOMA. Plasma CBG concentrations were not associated with risk factors at 9.5 years. Larger triceps skinfolds at birth were associated with lower plasma cortisol concentrations (β=-0.09 SD, 95% CI: -0.18,-0.003; p=0.03 adjusted for gestational age and sex). Birth weight (β=-0.12 SD, 95% CI: -0.21,-0.03; p=0.02) and length (β=-0.13 SD, 95% CI: -0.21,-0.05; p=0.002) were inversely related to plasma CBG concentrations.

Higher HPA axis activity is associated with higher cardio-metabolic risk factors even during childhood in India. Cortisol and CBG concentrations suggested a 'dampened' rather than 'heightened' cortisol effect in children who were smaller or adipose at birth. Dynamic testing of HPA axis activity (such as measuring stress responses) would be useful in investigating this further.

O-3iv-6

Cardiovascular Function in Adult Survivors of Severe Childhood Malnutrition. Ingrid A. Tennant[1,2], Alan T. Barnett[1,2], Jan Kips[3], Debbie S. Thompson[1], Michael S. Boyne[1], Edward E. Chung[4], Andrene P. Chung[4], Clive Osmond[1,5], Mark A. Hanson[6], Peter D. Gluckman[7], Patrick Segers[3], J. Kennedy Cruickshank[8], Terrence E. Forrester[1]. *[1]Tropical Medicine Research Institute, The University of the West Indies, Jamaica; [2]Department of Surgery, Radiology, Anaesthesia and Intensive Care, The University of the West Indies, Jamaica; [3]Institute Biomedical Technology, Ghent University, Belgium; [4]Department of Medicine, University of the West Indies, Jamaica; [5]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom; [6]DOHaD Division, School of Medicine, University of Southampton, United Kingdom; [7]UK Centre for Human Evolution, Adaptation and Disease, Liggins Institute, University of Auckland, New Zealand; [8]School of Epidemiology and Health Sciences, University of Manchester, United Kingdom.*

Severe childhood malnutrition can be oedematous (kwashiorkor) or non-oedematous (marasmus). These syndromes may be developmentally determined and effects may persist into adulthood. We hypothesized that cardiovascular structure and function would differ in adult survivors. Our aim was to compare measures of arterial stiffness, atherosclerosis and left ventricular function in survivors of kwashiorkor (SK) and marasmus (SM).

We recruited 29 adult SK and 25 SM (aged <50 years) originally treated in our metabolic ward. Anthropometry and blood pressure were measured. 2D-echocardiography, carotid and femoral ultrasound and tonometry of the brachial, radial and carotid arteries were performed. Cardiac output (CO), stroke volume (SV), carotid-femoral pulse wave velocity (PWV), carotid compliance, carotid and femoral intima-media thickness (IMT) and augmentation index were derived.

Mean (SD) age was 27.6 (7.9) years, 45% were women, and BMI was 23.4 (4.9) kg/m^2 with SK 2.5 kg/m^2 higher than SM (p=0.05). SK exceeded SM, expressed in mean (SE) standardized units, as follows: PWV 0.65 (0.25, p=0.01); augmentation index -0.07 (0.27); carotid compliance 0.12 (0.27); carotid IMT 0.38 (0.25); femoral IMT 0.02 (0.27); CO 0.34 (0.26) and SV 0.28 (0.27). Adjusted for BMI, the mean difference in PWV was 0.43 (0.21, p=0.05).

SK have greater arterial stiffness than SM, partially attenuated by their higher current BMI. Other cardiovascular measures were also substantially different in SK compared to SM, though not statistically so. If confirmed in larger samples, different types of severe malnutrition may be associated with divergent vascular outcomes in later life.

O-3v

Risk, Resilience, and Gene-Environment Interplay in Primates. Steven Suomi. *Invited Speaker, USA.*

O-3v-1

Developmental Programming of the Female Hypothalamic-Pituitary-Adrenal (HPA) Axis across Two Generations Following Dexamethasone (DEX) Administration in Ovine Pregnancy. Nathan M. Long[1], Peter W. Nathanielsz[2], Stephen P. Ford[1]. *[1]Center for the Study of Fetal Programming, University of Wyoming, WY, USA; [2]Center for Pregnancy and Newborn Research, University of Texas Health Sciences Center, TX, USA.*

Synthetic glucocorticoids (sGC) are routinely administered to women in risk of preterm labor. While there is accumulating evidence indicating that maternal sGC treatment programs offspring HPA axis activity, persistence of effects across multiple generations has not been evaluated in a precocial species.

F0 nulliparous ewes of similar age and weight were bred to a single fertile ram, and fed at NRC recommendations through gestation and lactation. At 0800h and 2000h on d 103 and d104 of gestation, ewes received an i.m. injection of 2 mg dexamethasone (DEX). Control (C) ewes received saline. Ewes lambed naturally. After weaning, female F1 lambs were maintained together and received NRC recommendations. At 22 ± 4 months of age, F1 ewes were naturally mated to a single fertile ram to produce the F2 lambs. DEX (n=6) and C (n=6) F1 ewes (3 ± 0.5 yrs of age) and DEX (n=6) and C F2 (n=6) ewe lambs (6.0 ± 0.3 months of age) underwent ACTH (0.2 µg/kg) and CRH/AVP (0.5 µg/kg and 0.1 µg/kg respectively) challenges. Cortisol response to ACTH challenge and cortisol and ACTH response during CRH/AVP challenge were analyzed using SAS.

. Body weights of DEX F1 ewes and DEX F2 lambs were less ($P < 0.05$) than C F1 ewes and C F2 lambs (65.1 ± 2.5 and 44.8 ± 2.4 vs. 72.2 ± 2.5 and 51.2 ± 2.4 kg, respectively). Baseline cortisol was higher ($P < 0.05$) while baseline ACTH was similar in DEX F1 ewes compared to F1 C ewes (1.8 ± 0.1 vs 0.8 ± 0.1 mg/dl and 30.1 ± 9.1 vs 36.8 ± 9.1 pg/ml, respectively). Baseline cortisol and ACTH were higher ($P < 0.05$) in DEX F2 lambs than C F2 lambs (1.8 ± 0.1 vs 0.9 ± 0.1 mg/dl and 56.2 ± 6.4 vs 35.4 ± 6.4 pg/ml, respectively). DEX F1 ewes and DEX F2 lambs had reduced ($P<0.05$) cortisol response post ACTH infusion compared to C F1 ewes and C F2 lambs. During the CRH/AVP challenge DEX F1 ewes and DEX F2 lambs had reduced ($P < 0.05$) post infusion cortisol and ACTH, resulting in a reduced ($P < 0.05$) area under the curve compared to C F1 ewes.

DEX administration reduced HPA axis activity, but increased baseline activity in female offspring. This programming effect carried over into the subsequent generation. Supported in part by NIH INBRE #P20RR016474.

O-3v-2

Early Life Programming of the HPA Axis: Effects of Maternal Stress. Helen C. Atkinson[1], Blagica Penova-Veselinovic[1], Q. W. Ang[1], J. Anke M. van Eekelen[2], Stephen J. Lye[3], Stephen G. Matthews[4], John P. Newnham[1], Craig E. Pennell[1]. *[1]School of Women's and Infants' Health, The Univeristy of Western Australia, WA, Australia; [2]Centre for Genetic Epidemiology, TICHR, WA, Australia; [3]Samuel Lunenfeld Research Institute, University of Toronto, Canada; [4]Department of Physiology, University of Toronto, Canada.*

An adverse *in utero* environment has been shown to influence the stress axis in later life. Birth weight is often used as a surrogate marker for an adverse *in utero* environment. Little is known about the relationship between exposure to antenatal and postnatal maternal stress and basal HPA axis function in adolescence. *The AIM of this study was to investigate the role of maternal stress in the association between birth weight and basal HPA activity in adolescence.*

The Western Australian Pregnancy (Raine) Cohort recruited 2900 pregnant women. Mothers reported life stress events experienced at 18 and 34 weeks gestation and then followed up when their children were one, two, three and five years of age. Basal HPA activity was assessed at 17-years. Awakening salivary samples were collected on three successive mornings for cortisol determination (salCORT). On the third morning a fasting blood sample was collected and the plasma analysed for cortisol (totalCORT), ACTH and CBG. Unbound cortisol (freeCORT) in plasma was calculated using the Coolen's equation. Multivariate regression analysis was used to investigate

associations between maternal stress and birth weight. Univariate regression analysis was used to investigate association between maternal stress and basal adolescent HPA activity.
In females there were no associations between maternal stress and either birth measures or basal HPA activity. In males there was a negative association between maternal stress during pregnancy (p=0.007) and birth-weight. This association was no longer significant if preterm babies (n=49) were excluded from the analysis. There were no associations between maternal stress during pregnancy and any of the measures of basal HPA activity in adolescence. In males there was an association between early life stress (combined for years 1,2,3 & 5) and salCORT in adolescence (p=0.034).
Stress during pregnancy does not appear to program basal activity of the HPA axis in late adolescence. The association between maternal stress and lower birth weight appears to be limited to preterm males. Stress during pre-school years, however, may program the basal HPA axis in late adolescence in males.

O-3v-3

Mineralocorticoid Receptor (MR)-Induced Gene Expression Pathways Implicated in Fetal Heart Growth Resulting from Increased Maternal Cortisol Levels. Elaine M. Richards[1], Maria B. Rabaglino[2], Xiaodi Feng[1], Maureen Keller-Wood[1]. *[1]Pharmacodynamics, University of Florida, Gainesville, FL, USA; [2]Physiology and Functional Genomics, University of Florida, Gainesville, FL, USA.*
Previous studies from our lab and others have shown that modest increases in maternal cortisol levels in sheep over a 10-day period from 120 to 130-days gestation (term=~147-days) resulted in increased fetal heart growth mediated by MR and resulting from increased cell proliferation, rather than hypertrophy or fibrosis. This increased growth occurred without changes in either fetal blood pressure or cortisol levels. Furthermore, RT-PCR analysis of genes previously shown to be altered during fetal heart growth revealed no significant differences under these conditions. In order to discover signaling pathways underlying this growth, microarray analysis followed by pathway analysis of the altered gene expression was conducted.
Expression levels of 15,000 sheep genes were analyzed using Agilent® ovine arrays in RNA isolated from left ventricles of four groups of fetal sheep; control (maternal cortisol vehicle and fetal heart vehicle infused); maternal cortisol infused (F) and fetal heart vehicle infused; F and fetal heart infused glucocorticoid receptor blocker(F+GRb); F with fetal heart infused MR blocker(F+MRb), (n=4/group).
Over 3,000 genes were expressed with significant difference among these groups but approximately 93 genes fit the pattern of significantly different expression with F and inhibition with MR blocker. Of these 18 were unidentified, but pathway analysis of the remainder using Webgestalt showed that the KEGG pathways depicting immune cell activation and metabolic pathways (alcohol metabolism) were significantly overrepresented. Also expression of collagens 3, 16 and 21 were altered.
This suggests that the increased maternal cortisol caused fetal heart growth patterns reminiscent of fibrotic heart growth in the adult heart (immune cell activation and collagen synthesis). It also (metabolic pathway) accelerated the timing of the switch from glucose as the major energy source for the heart to fatty acids, which occurs perinatally in normal sheep. These data suggest that sheep (and humans) born to mothers experiencing mild increases in circulating cortisol during late pregnancy could be predisposed to cardiovascular disease due to heart enlargement and activation of these genes.

O-3v-4

Body Mass Is Altered in F2-Generation Female Guinea Pigs after Chronic Stress during F0-Pregnancy. Hanna Schöpper, Teresa Klaus, Thomas Ruf, Susanne Huber. *Department of Integrative Biology and Evolution, Research Institute of Wildlife Ecology, University of Veterinary Medicine, Vienna, Austria.*
Chronic stress can have long-term programming effects that may even be transmitted to subsequent generations. Our aim was to investigate effects of chronic stress during F0-pregnancy on body weight development and reproductive maturation of the F2-generation in guinea pigs (*Cavia aperea f. porcellus*). Furthermore we examined whether maternal behavior (F1) during the first six days could have contributed to transmission.
Pregnant F0-females were subjected to high frequency strobe light twice a day (9-11am, 4-5pm; stressed females; n=7) once per week for the first two thirds of gestation. Control F0-females were left undisturbed. F2 offspring had either a mother (moPS, n=31), or a father (faPS, n=16) who had been prenatally stressed or both parents originated from control females (con/con, n=20). F2-offspring were weighed until day 68 in females and 124 in males.
We found that F2-offspring received similar levels of maternal behavior. The only significant difference was seen in moPS and faPS individuals receiving significantly less aggressive behavior than con/con (Kruskal-Wallis test: p=0.047). Body weight of F2-females was dependent on time, litter size, group and interactions of those factors (LME:time:litter size:group: $F_{4/1075}$=14.149, p<0.001, n=39). F2-females from small litters put on more weight than those from large litters, except moPS-females, which were heavier in bigger litters. Neither onset of estrus, nor body weight or age at first estrus were significantly different between groups (Kruskal-Wallis tests: p>0.05). In F2-males, body weight was not significantly different between groups, but differed between litter sizes over time (LME: time:litter size: $F_{1/961}$=29.431, p<0.001, n=28), F2-males from small litters growing faster than those from larger ones.
We conclude that chronic stress during F0-pregnancy influenced body weight development of F2-females, showing the strongest effect in large litters. As this effect is mainly seen in moPS-females, this points to maternal transmission. On the other hand, chronic stress during F0-pregancy did not significantly affect body weight of F2-males or reproductive maturation of F2-females. It seems as if maternal transmission of effects of prenatal stress to second generation offspring is primarily affecting female growth and might be modulated by less aggressive behavior of the dam.

O-3v-5

Anxiety and Depression in Severely Obese Pregnancy: Associations with Gestational Weight Gain and Birthweight. Rebecca M. Reynolds, Shareen Forbes, Nor Mohd-Shukri, Felicity H. Craighead, Rebecca N. Smith, Fiona C. Denison, Jane E. Norman. *Tommy's Centre for Maternal and Fetal Health, University of Edinburgh, United Kingdom.*
Obesity is associated with increased symptoms of anxiety and depression. We hypothesized that severe obesity in pregnancy is associated with adverse psychological health influencing gestational weight gain (GWG) and birthweight (BWT). We aimed to study mood and BWT among participants in a longitudinal study of severe obesity in pregnancy.
117 severely obese (BMI (mean(SEM)) 43.9(0.3) kg/m^2) and 62 lean (BMI 22.6(0.2) kg/m^2) pregnant women were recruited. Ethical approval and written, informed consent were obtained. Obese were advised about healthy eating and weight maintenance. Serial weight was recorded and GWG calculated between 16 and 36 weeks gestation. Women completed validated questionnaires to assess mood including Satisfaction with Life, Hospital Anxiety and Depression Scale (HADs) and Spielberger State and Trait Anxiety in early (12-20 weeks gestation) and late pregnancy (28-32 weeks gestation). Term BWT (>37 weeks gestation) was recorded (n=204).
Obese were significantly less satisfied with life than lean and had higher HADs depression and anxiety scores, and state and trait anxiety scores at both time points (all p<0.05). Findings remained significant after adjustment for social class.
Obese had less GWG than lean (5.5(0.9) vs 10.8(0.6) kg, p<0.05). 23% of obese had more GWG than Institute of Medicine guidelines. Offspring BWT was similar in obese and lean (3601(39.8) vs 3593(77.1), p=ns).
In lean, increased BWT was associated with higher BMI (r=0.68, p=0.002) and greater GWG (r=0.57, p=0.005). BWT was not related to BMI or GWG in obese. Higher HADs anxiety scores were associated with less GWG in lean (r=-0.38, p=0.01) but more GWG in obese (r=0.33, p=0.04). Increased state anxiety was associated with lower BWT in early pregnancy in both groups (p=0.03) with similar patterns in late pregnancy. BWT was not related to satisfaction or HADS scores. Findings remained significant after adjustment for gender, gestation, smoking, social class, parity and ethnicity.
Severely obese pregnant women have more symptoms of anxiety and depression and are less satisfied with life than lean. Increased anxiety in response to pregnancy is associated with lower BWT in all, but altered mood has differing associations with GWG in lean and obese. Understanding mood may help interventions to optimize GWG in severely obese women.

O-3v-6

Simultaneous Induction of 11β-HSD1 and H6PD Expression in Fetal Baboon Adipose Tissue by Prenatal Betamethasone Treatment. Chunming Guo, Leslie Myatt, Peter W. Nathanielsz, Kang Sun. *Center for Pregnancy and Newborn Research, Department of OB/GYN, University of Texas Health Science Center at San Antonio, TX, USA.*

Glucocorticoids are commonly administered to women threatening to deliver prematurely to enhance neonatal survival. However, persistent changes in offspring phenotype are debated. Accumulating evidence indicates that overexposure to glucocorticoids may predispose offspring to development of later life chronic diseases. Increased local regeneration of biologically active cortisol/corticosterone from their inactive counterparts by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in key metabolic tissues is believed to be fundamental to this developmental programming. 11β-HSD1 reductase activity is dependent on the availability of the co-factor NADPH produced by hexose-6-phosphate dehydrogenase (H6PD). We hypothesized that fetal exposure to glucocorticoids at levels higher than appropriate for the current stage of maturation would have a persistent effect on 11β-HSD1 and H6PD in adipose tissue.

To mimic a commonly administered human clinical regimen we administered betamethasone (175 μg/kg, equivalent to the human clinical dose) or vehicle once daily i.m at 8:00 am at 111, 112, 118, 119, 125, and 126 dG to pregnant baboons (n=6-7 animals per group). Fetal peri-renal adipose tissue was collected at 175 dG (term: 184 days). qRT-PCR and Western blotting were performed to measure expression of 11β-HSD1 and H6PD mRNA and protein.

Prenatal betamethasone treatment increased 11β-HSD1 and H6PD mRNA expression by 2.2 and 2.3 fold respectively ($P < 0.05$) in fetal adipose tissue. In parallel betamethasone treatment increased 11β-HSD1 and H6PD protein levels by 1.6 and 1.4 fold respectively ($P < 0.05$ or $P < 0.01$). Using a mouse preadipocyte cell line 3T3-L1, we evaluated glucocorticoid induction of 11β-HSD1 and H6PD mRNA and protein expression in vitro. Corticosterone (0.1 μM) significantly induced expression of 11β-HSD1 and H6PD mRNA and protein, which was attenuated by the glucocorticoid receptor antagonist RU486 (1 μM).

We have demonstrated that weekly courses of fetal exposure to glucocorticoids simultaneously induced 11β-HSD1 and H6PD expression in fetal adipose tissue. This would increase catalytic activity of 11β-HSD1 in the reductase direction and potentially result in increased local regeneration of biologically active glucocorticoids in the adipose tissue that may program later life obesity and metabolic dysfunction.

O-3vi-A

Perinatal Programming of Childhood Obesity and Type 2 Diabetes - A Vicious Cycle. Dana Dabelea. *Invited Speaker, USA.*

O-3vi-B

Cardiometabolic Complications of Pregnancy. Ellen Seely. *Invited Speaker, USA.*

O-3vi-1

Gestationally Programmed Non-Alcoholic Fatty Liver in Intrauterine Growth Restricted Fetuses: Upregulated Hepatic Lipogenesis Prior to Obesity. Makiko Yamada[1], Diana Wolfe[1], Guang Han[1], Samuel W. French[2], Michael G. Ross[1], Mina Desai[1]. *[1]Obstetrics & Gynecology, David Geffen School of Medicine at UCLA and Los Angeles Biomedical Research Institute, CA, USA; [2]Pathology, Harbor-UCLA Medical Center, CA, USA.*

Intrauterine growth restricted newborns (IUGR) have increased risk of adult metabolic syndrome and non-alcoholic fatty liver disease (NAFLD). We have simulated this scenario using a rat model of maternal food restriction that results in IUGR newborns with subsequent development of adult obesity and fatty liver. However, it is unclear whether IUGR mediated NAFLD is developmentally programmed or secondary to obesity. To address this, we studied hepatic lipid deposition prior to onset of obesity in IUGR near-term fetuses and newborns. As the critical underlying mechanism(s) for NAFLD involves de novo lipogenesis and/or mitochondrial fat oxidation, we determined the expression of lipogenic transcription factor (sterol regulatory element-binding protein, SREBP1c) and lipogenic enzyme (fatty acid synthase, FAS), and the morphology of hepatic mitochondria.

Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to 21 to produce IUGR newborns. On embryonic day 20 (e20) and postnatal age (p1), livers were collected for lipid quantification (osmium stain), mitochondrial morphology (electron microscopy) and protein expression (Western Blot) of SREBP1c and FAS.

At e20, hepatic lipid deposition was significantly increased in IUGR fetuses as compared to Controls (0.98±0.43 vs. 0.04±0.01 % lipid/liver parenchyma; $p<0.01$), where it was virtually undetectable. In conjunction, IUGR fetuses showed significant upregulation of SREBP1c (1.7-fold) and FAS (1.8-fold). However, there was no evidence of abnormality in mitochondrial shape in IUGR livers. At p1, both IUGR and Control newborns demonstrated a marked, though comparable, increase in hepatic lipid content, though IUGR newborns expressed continued upregulation of SREBP1c (1.3-fold) and FAS (1.4-fold).

Enhanced hepatic fatty accumulation together with upregulated lipogenesis in IUGR fetuses suggest that NAFLD is programmed, independent of adult obesity. Oral intake of high fat rat milk resulted in an acute, marked increase in hepatic lipid content in both IUGR and Control newborns. However, the persistent upregulation of SREBP1 and FAS indicates that IUGR offspring are predisposed to hepatic fat accumulation following weaning.

O-3vi-2

The Impact of Neonatal Breastfeeding on the Childhood BMI Growth Trajectory of Children Exposed to Diabetes In Utero: The EPOCH Study. Tessa L. Crume[1], Lorranie Ogden[1], Elizabeth J. Mayer-Davis[2], Richard F. Hamman[1], Dana Dabelea[1]. *[1]Epidemiology, Colorado School of Public Health, University of Colorado Denver, CO, USA; [2]Nutrition and Medicine, University of North Carolina at Chapel Hill, NC, USA.*

To evaluate whether neonatal breastfeeding at recommended levels can attenuate the increased childhood BMI growth trajectory and accelerated growth velocity associated with *in utero* exposure to maternal diabetes.

Mixed linear effects models were constructed to assess differences in BMI and BMI growth velocity from birth to 13 years of age for 94 subjects exposed to gestational diabetes *in utero* and 409 unexposed subjects. A measure of breast milk dose was derived from maternal self-report to categorize breastfeeding status as low (<6) and adequate (≥6 breast milk-months). A stratified analysis was conducted to determine if adequate levels of neonatal breastfeeding attenuated the risk of accelerated growth in infancy and childhood associated with exposure to maternal diabetes. All models were adjusted for sex and race/ethnicity.

Among subjects who had low neonatal breastfeeding status (<6 breast milk-months), exposure to maternal diabetes *in utero* was associated with a different BMI growth curve during childhood ($p=0.02$), average higher BMI among exposed subjects from 27 months to 13 years ($p=0.05$) and increased growth velocity among exposed subjects between 10 and 13 years of age ($p=0.02$) (Figure1). However, among subjects who were adequately breastfeed (≥6 breast milk-months), there was not a significant effect of exposure on the BMI growth trajectory during infancy ($p=0.56$) or childhood ($p=0.27$). In addition, increased BMI growth velocity was not detected at any point along the infancy or childhood growth curve. These findings remained significant after additional adjustment for differences in Tanner stage, daily energy intake and physical activity patterns.

Breastfeeding in the early postnatal period may represent a critical opportunity to reduce the risk of accelerated childhood BMI growth among youth exposed to a diabetic pregnancy.

O-3vi-3

A Latent Variable Analysis of Birth Weight and Childhood Obesity. Timothy B. Gage[1,2], Fu Fang[1], Erin K. O'Neil[1], A. G. DiRienzo[2]. *[1]Department of Anthropology, University at Albany, NY, USA; [2]Epidemiology and Biostatistics, University at Albany, NY, USA.*

Size at birth is inversely associated with the risk of chronic diseases later in life. However, analyses using conventional statistical approaches have shown that obesity, a risk factor for chronic diseases, generally increases with birth weight. Several studies have concluded that maternal size, not fetal programming, underlies the observed obesity by birth weight relationship.

Covariate Density Defined mixture of regressions (CDDmr) was developed to study birth weight and infant mortality while accounting for significant, yet hidden, heterogeneity in mortality. It identifies two latent subpopulations interpreted as undergoing "normal" and "compromised" fetal development and resolves the "pediatric paradox".
Here we use CDDmr to study birth weight and childhood obesity while controlling for maternal size.
CDDmr is applied to the 1958 British Longitudinal Birth Cohort by sex. After controlling for maternal weight, body mass index (BMI) at age seven is modeled as a mixture of two polynomial regressions by birth weight with weights determined by the birth weight density, which is a mixture of two Gaussians.
The results are consistent with our previous analyses of birth weight and infant mortality. There is significant heterogeneity in BMI. The parsimonious model predicts that, after correcting for maternal weight, mean BMI increases linearly with birth weight (grams), coefficient 4.1 x 10-4, among "normal" births; among "compromised" births, mean BMI is a convex quadratic function of birth weight. Compared to their "normal" peers, "compromised" births display significantly higher BMI across all observed birth weights and thus have faster post-birth growth rates. Maternal age adjusted mean BMI is 15.6 & 15.8 kg/m2 for "normal" girls and boys, respectively, while "compromised" births have an average BMI of 18.4 & 17.7 kg/m2 for girls and boys, respectively. Though only 11% of the total births are "compromised", they account for 48% & 37% of the overweight, and 93% & 87% of the obese girls and boys at age 7, respectively.
From birth weight alone, CDDmr identifies a latent subpopulation of fetal-programmed births, which have experienced faster postnatal growth, and accounts for most obese children at age 7. CDDmr, which incorporates latent variable techniques, may prove to be a useful statistical method for studying fetal programming in general.

O-3vi-4

DNA Methylation Patterns in Cord Blood DNA Predict Body Size in Childhood. Caroline L. Relton[1], Alexandra Groom[1], Beate St Pourcain[2], Adrian E. Sayers[2], Daniel C. Swan[3], Nicholas D. Embleton[4,5], Mark S. Pearce[5], Sue M. Ring[2], Kate Northstone[2], Jon H. Tobias[6], Andy R. Ness[7], Seif O. Shaheen[8], George Davey Smith[2]. *[1]HNRC, Institute of Genetic Medicine, Newcastle University, United Kingdom; [2]MRC Centre for Causal Analyses in Transaltional Epidemiology, University of Bristol, United Kingdom; [3]Bioinformatic Support Unit, Newcastle University, United Kingdom; [4]Newcastle Neonatal Service, Royal Victoria Infirmary, United Kingdom; [5]Institute of Health and Society, Newcastle University, United Kingdom; [6]Southmead Hospital, Bristol, United Kingdom; [7]School of Dental Sciences, Univeristy of Bristol, United Kingdom; [8]School of Medicine and Dentistry, Queen Mary University London, United Kingdom.*
Epigenetic markings acquired in early life may have phenotypic consequences later in development via transcriptional regulation with relevance to the developmental origins of obesity. This study investigated whether DNA methylation levels at birth predicts body size later in childhood.
A study design involving two childhood cohorts was used to conduct transcription profiling followed by DNA methylation analysis in peripheral blood. Gene expression analysis was undertaken in 24 individuals whose biological samples and clinical data were collected at a mean (SD) age of 12.35 (0.95) years, the upper and lower tertiles of body mass index (BMI) were compared with a mean (SD) BMI difference of 9.86 (2.37) kg/m^2. This generated a panel of differentially expressed genes and DNA methylation analysis was then undertaken in cord blood DNA in 178 individuals with body composition data collected at a mean (SD) age of 9.83 (0.23) years.
29 differentially expressed genes (>1.2-fold and $p<10^{-4}$) were analysed to determine DNA methylation levels at 1-3 sites per gene. Five genes were unmethylated and DNA methylation in the remaining 24 genes was analysed using linear regression with bootstrapping. Methylation in nine of the 24 (37.5%) genes studied was associated with at least one index of body composition (BMI, fat mass, lean mass, height) at age nine years, although only one of these associations remained after correction for multiple testing (*ALPL*).
DNA methylation in differentially expressed genes predicts later body size in childhood, suggesting that processes initiated during the antenatal period are involved in the determination of later body composition. This provides evidence of epigenetic perturbations existing many years before phenotype ascertainment.

O-3vi-5

Intrauterine Influences on Offspring Obesity in Prepubertal Children. Oana Maftei[1,2], Melissa J. Whitrow[1,2], Vivienne M. Moore[1,2], Michael J. Davies[1]. *[1]Research Centre for the Early Origins of Health and Disease, Robinson Institute, The University of Adelaide, South Australia, Australia; [2]Discipline of Public Health, The University of Adelaide, South Australia, Australia.*
To examine associations of maternal obesity at conception, glucose intolerance during pregnancy across the entire spectrum, and gestational weight gain with offspring BMI z-score in 9.5 year-old children.
We analysed data from a representative, prospective birth cohort study (Generation 1 Study, n=557) in Adelaide, South Australia, recruited during 1998-2000. At 9.5 years, 443 children (80% of original cohort) took part in the study, with 163 providing a fasting blood sample. The main intrauterine exposures were: (1) maternal pre-pregnancy BMI (self-reported pre-pregnancy weight and height measured in early pregnancy); (2) glucose tolerance during pregnancy: normal glucose tolerance (NGT); borderline gestational glucose intolerance (BGGI), defined by a positive oral glucose challenge test (OGCT) and a negative oral glucose tolerance test (OGTT); and gestational diabetes (GD), defined by positive OGCT and OGTT; (3) gestational weight gain (GWG). The primary outcome was child BMI z-score. Potential confounders were maternal age, parity, smoking, pregnancy-induced hypertension, and education. Associations between each intrauterine exposure and child BMI z-score at 9.5 years were assessed using linear regression models.
Child BMI z-score at 9.5 years was positively associated with maternal pre-pregnancy BMI, and this was robust to confounder adjustment (for each 1 kg/m^2 increase in pre-pregnancy BMI, child BMI z-score increased by 0.08, 95% CI 0.06-0.10, p<0.0001). Mean BMI z-score of children exposed to either GD or BGGI was not significantly different to the BMI z-score of children whose mothers maintained NGT during pregnancy, both in unadjusted and fully adjusted models. There was no association between GWG and child BMI z-score in the unadjusted model until adjustment for pre-pregnancy BMI. In the fully adjusted model, child BMI z-score increased by 0.032 (95% CI 0.007-0.057, p=0.014) for each 1 kg increase in GWG.
Among the intrauterine factors considered in this study, maternal pre-pregnancy BMI was the strongest predictor of child BMI z-score. These findings provide support for transgenerational acceleration in the prevalence of obesity and suggest the importance of optimizing maternal weight before conception for obesity prevention in the offspring.