Antenatal maternal anxiety is associated with impairments in cognitive control

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A. BASIC SCIENCE, REGULATION OF SOMATIC GROWTH, EPIGENETICS

A-01
Histone acetylation is necessary for alveolar septation in preterm lambs with evolving chronic lung disease

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Objective: Chronic lung disease (CLD) of prematurity is characterized by DNA hypermethylation and H3 hypoacetylation. Long-term changes in gene expression and phenotypic changes suggest persistent changes in gene expression by altering determinants of chromatin structure. We hypothesized that disruption of histone hypoacetylation will permit alveolar formation.
Materials/methods: Preterm lambs (~132 days gestation; term ~148 days), treated with antenatal steroids and postnatal surfactant, were managed by MV for 3 days and treated daily with valproic acid (VPA; 25 mg/kg, i.v.; $n=4$), trichostatin A (TSA; 0.10 mg/kg, i.v.; $n=4$), or vehicle ($n=4$). The positive gold-standard was management by nasal CPAP ($n=4$). At the end of 3 days, the lungs were analyzed morphometrically.
Results: VPA-treated and TSA-treated preterm lambs had more advanced alveolar formation than vehicle controls, as shown by morphometric measurements of radial alveolar count (6 ± 2 and 6 ± 1 vs. 2 ± 1, respectively; $p<0.05$), secondary septal volume density (11 ± 3 and 10 ± 3 vs. 5 ± 2, respectively; $p<0.05$), and alveolar wall thickness (2.89 ± 0.07 and 2.97 ± 0.08 vs. 3.88 ± 0.10, respectively; $p<0.05$). Alveolar formation in the VPA- or TSA-treated lambs was comparable to that in nasal CPAP-managed preterm lambs.
Conclusions: Management of preterm lambs with MV leads to alveolar simplification. Treating preterm lambs with valproic acid or trichostatin A, histone deacetylase inhibitors, permitted alveolar formation. We conclude that histone acetylation, which affects patterns of gene expression, is necessary for alveolar formation (CHRC, HD01410, HL62875, HL56401).

Disclosure: Was this work supported by industry? No.

A-02
Histone acetylation and methylation are altered in the hippocampus of preterm lambs managed by mechanical ventilation

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Objective: Mechanical ventilation (MV) of the premature human infant is associated with brain injury. In sheep, we have shown that MV injures the hippocampus. In other models, our group has shown that hippocampal injury is

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associated with epigenetically determined changes in gene expression. We hypothesized that histone acetylation and methylation in the hippocampus will be affected by MV of preterm lambs.
Materials/methods: Preterm lambs (~132 days gestation, term ~148 days), treated with antenatal steroids and postnatal surfactant, were managed by MV or nasal continuous positive airway pressure (CPAP) ($n=4$ each). Fetal lambs served as gestation controls ($n=4$). At the end of 3 days, the hippocampus was isolated and analyzed by immunoblot for acetylated H3K9, dimethyl H3K4, and trimethyl H3K4.
Results: Homogenates of the hippocampus of the MV group had significantly more acetylated H3K9, dimethyl H3K4, and trimethyl H3K4 protein than the nasal CPAP and the fetal control groups.
Conclusion: Management of preterm lambs with MV leads to altered acetylation of H3K9 and methylation of H3K4 in the hippocampus. We speculate that a specific subset of genes is expressed in the hippocampus due to MV-induced changes that lead to brain injury (CHRC, HD01410, HL62875, HL56401).

Disclosure: Was this work supported by industry? No.

A-03
Accelerated kidney growth and glomerular hyperfiltration in microswine offspring of protein restricted sows

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Microswine offspring of maternal protein restriction (MPR) exhibit early proportional reduction of body (BWt) and kidney Wt (KWt), followed by accelerated growth from 6 to $\geq$13 weeks. Our **objective** was to assess kidney growth pattern and function in juvenile offspring.
Methods: Time-mated sows were exposed to isocaloric MPR (1% vs. 14%) in last 1/3 of gestation +2 weeks (period of nephrogenesis). At 3—5 months of age, GFR, ERPF (plasma disappearance of insulin, PAH) in awake offspring and KWt, BWt and length (lgth) were measured at harvest.
Results: KidWt/BWt ratio was higher in LPO: 6.2 ± 0.8 g/kg ($n=11$) vs. 5.5 ± 0.7 in NPO ($n=13$), $p<0.04$. Higher KidWt/KidLgth in LPO (7.8 vs. 6.8, $p=0.05$) did not suggest thinness. GFR in LPO was normal (5.46 ± 1.61 cc/kg, $n=12$ vs. 4.87 ± 1.27 in NPO, $n=9$, $p=$NS); ERPF was reduced: 24.66 ± 7.54 in LPO ($n=10$) vs. 41.77 ± 9.45 in NPO ($n=7$), $p<0.001$. Filtration fraction was significantly increased: 0.24 ± 0.07 in LPO ($n=10$) vs. 0.13 ± 0.05 in NPO ($n=5$), $p<0.01$.
Conclusions: LPO kidney size is reduced in proportion to body weight reduction at 2 weeks, but kidney size increases faster than body size during accelerated compensatory growth. At 3—5 months, reduced ERPF with glomerular hyperfiltration in LPO suggest compensation for low nephron number. Accelerated kidney growth relative to body growth may reflect a primary renal growth stimulus independently of metabolic load; AngII, via activated NADPH oxidase pathways, may contribute.

Disclosure: Was this work supported by industry? No.

A-04
Immunohistochemical localization of the stem cell antibody TRA-1-81 in the fetal baboon kidney

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Objective: TRA-1-81 is a marker of undifferentiated human embryonic stem cells. This study examined localization of the TRA-1-81 antibody in primate kidneys from fetal baboons during normal gestation and from pre-term baboons ventilated after delivery.
Materials/methods: All animal handling and deliveries were performed at the Southwest Foundation for Biomedical Research, San Antonio, Texas. Fixed kidneys from baboons delivered at 125, 140, 175, and 185 days gestation (term ~185 days) and from baboons prematurely delivered at 125 days gestation and ventilated for 6, 14, and 21 days postnatally were used. TRA-1-81 was localized in 5 μm deparaffinized sections using a monoclonal anti-TRA-1-81 primary antibody and an anti-mouse secondary antibody conjugated to horseradish peroxidase. The sections were subsequently treated with 3′3-diaminobenzidine tetrachloride and counterstained with haematoxylin.
Results: In fetal kidneys at 125 and 140 days gestation and in pre-term kidneys at postnatal days 6 and 14, TRA-1-81 localized to the metanephric mesenchyme of the nephrogenic zone in the outer cortex and also to the collecting ducts. At 175 and 185 days in normal gestation and at postnatal day 21 in pre-term kidneys, the nephrogenic zone was no longer present; positive staining localized to the epithelium of the glomerular capillaries, the renal tubules, and the collecting ducts.
Conclusion: In both fetal and pre-term kidneys, the metanephric mesenchyme appears to contain undifferentiated kidney precursor cells marked by TRA-1-81. Importantly, at completion of nephrogenesis, the epithelium of the glomerular capillaries, tubules, and collecting ducts may continue to be a source of undifferentiated kidney precursor cells.

Disclosure: Was this work supported by industry? No.

A-05
A high unsaturated fat, high protein, and low carbohydrate diet during pregnancy and lactation modulates peripheral adipokine receptor gene expression in adult mouse offspring

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Objectives: Leptin and adiponectin are adipocytokines that regulate metabolism. Leptin modulates appetite via its receptor (Ob-Rb) in the hypothalamus, whereas adiponectin regulates insulin sensitivity via two receptor subtypes (AdipoR1, AdipoR2) in insulin sensitive tissues. The aim of

this study was to characterise AdipoR, AdipoR2, and Ob-Rb gene expression in peripheral tissues of adult mouse offspring exposed to maternal high fat-high protein diet (HFP) *in utero* and during lactation.
Methods: Female Balb/C mice were either fed a high fat-high protein diet (36.8% CHO, 32% lipid, 28% protein; $n=9$) or standard chow (68.8% CHO, 10% lipid, 18% protein; $n=6$) 6 weeks prior to conception through to pregnancy and lactation. Weaned offspring were fed the chow diet until adulthood. At 8 weeks old, offspring were killed, tissues collected and analyzed for changes in gene transcript levels by PCR-based techniques.
Results: In adipose tissue, AdipoR1, AdipoR2, and Ob-Rb mRNA levels were 40%, 45%, and 207% higher respectively in males vs. females ($p<0.05$). Moreover in all offspring, AdipoR2 mRNA levels were 10-fold higher than AdipoR1 transcripts in this tissue ($p<0.001$). In HFP female offspring, but not in males, AdipoR1 and AdipoR2 levels increased by 48% and 26% respectively vs. the chow group. In the liver, different patterns of gene expression were also observed between sexes.
Conclusions: Prenatal exposure to a HFP diet modulates adipocytokine receptor gene expression in adult offspring. Different patterns of gene expression were observed between males and females. These data suggest adipocytokine receptor expression is developmentally regulated in a sex-specific manner.

Disclosure: Was this work supported by industry? No.

A-06
Feeding a protein-restricted diet during pregnancy in the rat induces altered gene promoter methylation in the liver of the F1 and F2 offspring

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Background: There is evidence in humans and experimental animals for non-genomic transmission of induced phenotypes between generations. Induction of an altered phenotype in the F_1 generation by feeding a protein-restricted (PR) diet to pregnant rats involves altered epigenetic regulation of specific genes. We investigated whether altered epigenetic regulation of hepatic PPARα and glucocorticoid receptor (GR) promoters induced in the F_1 generation by prenatal under-nutrition is passed to the F_2 offspring.
Methods: Rats (F_0) were fed a control (18% protein) or PR (9% protein) diet throughout pregnancy and chow during lactation. F_1 females were mated and fed chow throughout pregnancy and lactation. F_1 and F_2 offspring were fed chow. Male offspring from the F_1 and F_2 generations ($n=6$ per F_0 dietary group) were killed at day 80. Methylation of the hepatic PPARα and GR promoters was determined by methylation-sensitive real-time PCR. mRNA expression was measured by real-time RT-PCR.
Results: Methylation of the hepatic PPARα and GR promoters was lower (8% to 11%, $p<0.05$) in the F_1 and F_2 PR offspring. There were trends towards higher PPARα and GR expression in the PR offspring.
Conclusion: Altered promoter methylation and expression induced in the F_1 generation by prenatal under-nutrition can be transmitted to at least the F_2 generation. This suggests a mechanism for transmission of phenotypes induced by maternal nutritional constraint between generations of offspring.

Disclosure: Was this work supported by industry? No.

A-07
Quantitative analysis of the methylation status of the hepatic PPAR-alpha promoter CPG island by pyrosequencing in offspring of rats fed a protein-restricted diet during pregnancy

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Background: Nutritional constraint during gestation induces persistent phenotypic changes to the offspring which are associated with increased risk of chronic non-communicable disease. Persistent alterations to the phenotype of the offspring imply stable changes to transcription. Assessment of DNA methylation using methylation sensitive real-time PCR (MSPCR) showed that feeding pregnant rats a protein-restricted (PR) diet induces increased hepatic PPARα expression in the adult offspring by decreased methylation of the PPARα promoter. The aim of this study was to confirm these methylation changes by sequencing the CpG island in the PPARα promoter and to determine the methylation status of individual CpG dinucleotides.
Methods: Rats were fed control (18% protein) or PR diets (9% protein) during pregnancy and chow throughout lactation. Pups were weaned at 28 days and killed at 34 days. DNA was extracted from 5 livers/group, modified by bisulphite treatment and amplified by PCR. PCR products were isolated and analysed by pyrosequencing.
Results: Overall PPARα promoter methylation was lower (46%, $p<0.001$) in the PR offspring compared to controls. Individual CpG dinucleotides showed differential hypomethylation.
Conclusion: These findings confirm our previous observations from analyses using MSPCR. The differential hypomethylation of individual CpGs suggests the possibility that, in addition to an increase in total expression, induction of an altered phenotype may involve subtle changes to the interactions between gene promoters and DNA binding proteins.

Disclosure: Was this work supported by industry? No.

A-08
The effect of prenatal under-nutrition on the expression of DNA methyltransferases and methyl CPG binding protein 2 in the liver after weaning

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Background: Induction of an altered metabolic phenotype in the offspring of rats fed a protein-restricted (PR) diet during pregnancy involves hypomethylation and increased

expression of the hepatic glucocorticoid receptor (GR) and PPARα promoters. To determine the mechanism responsible for promoter hypomethylation we investigated the effect of feeding a PR diet to pregnant rats on the expression of DNA methyltransferase (DMNT)-1 which maintains DNA methylation during mitosis, DNMT 3a and 3b which catalyse DNA methylation *de novo*, the DNA demethylase MBD2 and the methyl CpG binding protein (MeCP)-2 which recruits enzymes that regulate covalent histone modifications to methylated DNA in the liver of the adult offspring.
Methods: Rats were fed either a control or PR diet from conception to delivery, and chow during lactation. Offspring were weaned onto chow at postnatal day 28 and killed at postnatal day 34. mRNA expression was determined by real-time quantitative RT PCR.
Results: There was no effect of prenatal under-nutrition on the expression of DNMT 3a or 3b, or on the expression of MBD2. DNMT1 expression was significantly lower (17%, $p<0.05$) and MeCP2 expression was reduced (28.6%, $p<0.05$) in the PR offspring vs. controls.
Discussion: These results suggest that prenatal under-nutrition induces hypomethylation of the PPARα and GR promoters by reducing the capacity of DNMT1 to methylate hemimethylated DNA during mitosis. Thus induction of an altered phenotype by prenatal under-nutrition may be the result of impaired DNMT1 activity. Lower MeCP2 expression, together with hypomethylation of CpGs, may facilitate histone acetylation leading to increased transcription.

Disclosure: Was this work supported by industry? No.

A-09
The effect of prenatal under-nutrition, neonatal leptin exposure, and post-weaning fat intake on the expression of genes associated with lipid-metabolism in adipose tissue in female rats

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Background: Nutritional constraint during early life is associated with increased risk of disease such as obesity in later life. In rats, a phenotype characterised by obesity is induced by prenatal under-nutrition and consumption of a high-fat post-weaning diet (PWD), but is reversed by neonatal leptin treatment. To investigate mechanisms, we measured the expression of genes associated with lipid metabolism in adipose tissue in adult offspring following maternal under-nutrition during pregnancy, neonatal leptin administration, and increased dietary fat intake after weaning.
Methods: Pregnant Wistar rats were fed either *ad libitum* or 30% of *ad libitum* intake. Leptin or saline was administered to female offspring between postnatal days 3 to 13. Offspring were weaned onto a high fat (45% energy) or low fat (21% energy) PWD and killed at 170 days ($n=8$ per group). PPARα and γ2, carnitine: palmitoyl transferase (CPT)-1, acyl-CoA oxidase (AOX), and lipoprotein lipase (LPL) mRNA expression was measured by real-time RT PCR.
Results: There was no effect of prenatal nutrition or PWD on gene expression. There was a significant effect of leptin treatment ($p<0.0001$) on the expression of all of the genes measured. Leptin administration increased AOX and PPARγ$_2$ expression, while PPARα, CPT-1, and LPL expression was increased in the offspring which received neonatal leptin and a high fat PWD.
Conclusion: These results show for the first time that neonatal leptin exposure induces an altered metabolic phenotype in adipose tissue which can be modified in a gene-specific manner by the PWD and persists in adult animals.

Disclosure: Was this work supported by industry? No.

A-10
Adenosine receptor A2A stimulation decreases nitric oxide synthesis in placental microvascular endothelial cells from women with preeclampsia and intrauterine growth restriction

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Preeclampsia (PE) and intrauterine growth restriction (IUGR) are associated with endothelial placental dysfunction.
Objective: To study the nitric oxide synthase (NOS) activity and adenosine receptor A_{2A} stimulation in placental microvascular endothelial cell (hPMEC) from normal (N), PE, and IUGR pregnancies.
Methods: Placentae from N, PE, PE+IUGR, and IUGR were collected after delivery. hPMEC were isolated from fetal side of placentae and an enriched culture of endothelial cells was obtained using CD31 covered magnetic beads. Characterization of hPMEC was performed according to standard procedures. NOS activity was measured by L-[^{3}H]citrulline formation in the presence of *N*-nitro-L-arginine methyl ester (NOS inhibitor, L-NAME), A_{2A} selective agonist CGS-21680, and antagonist ZM-241385.
Results: Basal NOS activity was higher in PE and PE+IUGR compared with normal or IUGR (0.34 ± 0.19 and 0.46 ± 0.12 vs. 0.11 ± 0.04 and 0.10 ± 0.07 pmol/μg protein/30 min, respectively). CGS-2168 decrease NOS activity in pathological groups ($p<0.05$) but unaffected the normal pregnancy (0.012 ± 0.07 pmol/μg protein/30 min). These effects were blocked by ZM-241385.
Conclusions: NO synthesis is increased in hPMEC from preeclamptic pregnancies as a compensatory mechanism to the increased vascular resistance observed in this disease. IUGR appears as an independent pathological condition with normal NO synthesis. Adenosine receptor A_{2A} could modulate NO synthesis in placental vessels affecting the normal development observed in preeclamptic and IUGR complicated pregnancies.
Support: FONDECYT 1030781, 1030607 (Chile). C.E. holds a PhD-MECESUP (Chile) fellowship.

Disclosure: Was this work supported by industry? No.

A-11
Inhibition of L-arginine transport and nitric oxide activity in human umbilical vein endothelium of newborn with intrauterine growth restriction: role of hypoxia and PKC

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Intrauterine growth restriction (IUGR) is associated with hypoxia and vascular disorders later in life. In human umbilical vein endothelium (HUVEC) L-arginine transport is mediated by system y^+/CATs (Cationic Amino acid Transporters) and nitric oxide (NO) derives from conversion of L-arginine to L-citrulline via endothelial NO synthase (eNOS).
Objective: We studied the effect of hypoxia and the role of PKC on L-arginine transport and NO synthesis in HUVEC from foetuses with IUGR.
Methods: HUVEC cultured in M-199 were exposed (0—24 h) to normoxia (5% O_2, ~35 mm Hg PO_2) or hypoxia (2% O_2, ~15 mm Hg). L-Arginine transport was determined in presence or absence of PKC inhibitors. hCAT-1, hCAT-2B, and eNOS mRNA were quantified by real-time PCR. eNOS activity was determined by L-[^{3}H]citrulline formation, total and phosphorylated eNOS (Ser^{1177}), and PKC (α/βII) proteins were detected by Western blot.
Results: Maximal transport velocity (V_{max}) for L-arginine transport was reduced in IUGR (28%) and hypoxia (44%). Hypoxia effect was blocked by calphostin C (PKC inhibitor) in normal and IUGR cells. hCAT-1, hCAT-2B, and eNOS mRNA levels were reduced in IUGR and hypoxia. However, total eNOS and PKCα protein levels were increased in IUGR (1.7-fold) and hypoxia (1.3-fold), while phosphorylated eNOS at Ser^{1177} was reduced in normal and IUGR cells under hypoxia. NOS activity was reduced in IUGR (67%) and hypoxia (80%).
Conclusion: IUGR and hypoxia reduced L-arginine/NO pathway activity may result from lower expression and activity of hCAT-1, hCAT-2B, and eNOS, involving a NO-dependent activation of PKC in HUVEC. Support: FONDECYT 1030607, 1030781.

Disclosure: Was this work supported by industry? No.

A-12
Does the maternal micronutrient deficiency (Cu or Zn or vitamin E) modulates the expression of cardiac 11βhydroxysteroid dehydrogenase type-II and free radicals scavenging enzymes levels and per se predispose the offspring to hypertension in their adult life?

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Background: Epidemiological evidence suggests that some adult disorders like hypertension, insulin resistance syndrome and disease associated with it originate in fetal life. Maternal under-nutrition is hypothesized to predispose the offspring to disease in adult life. The relevance of maternal macronutrient deficiency has been well studied but not that of micronutrients.
Hypothesis: We hypothesize that chronic maternal dietary mineral (Cu or Zn or vitamin E) restriction modulates the expression of fetal cardiac 11βhydroxysteroid dehydrogenase-2 (11 β HSD-2) and free radical scavenging enzyme levels and per se individually alters the offspring's cardiac function and predispose the individual to hypertension.
Methods: Female weaning Swiss albino mice received a control diet (based on the American Institute of Nutrition AIN-93G diet) ($n=20$) or a 50% vitamin E-restricted diet ($n=20$) or 50% zinc-restricted diet ($n=20$) or 50% copper-restricted diet ($n=20$) and they were provided with demonized water for 12 weeks and mated with control males. Pups born to the dams on the restricted diet were weaned on to the respective restricted diet.
Functional physiology tests: At the end of 20 weeks of feeding of the offspring, various cardiac function tests were performed in the offspring.
Molecular analysis: Fetal cardiac samples were used for Western blot analysis of 11βhydroxysteroid dehydrogenase-2, super oxide dismutase, glutathione peroxidase, glutathione reductase, catalase in both control and micronutrient deficiency groups.
Results: Body weight and crown rump length were significantly ($p<0.001$) reduced in offspring, from dams fed micronutrient diet compared with control group. The micronutrient-restricted (Cu or Zn or Vit E) mice had significantly impaired cardiac functions and found hypertensive compared with control group. Western blot analysis of 11 β HSD-2 expressions, super oxide dismutase, glutathione peroxidase, glutathione reductase, and catalase in fetal cardiac tissues were downregulated in all the restricted diet fed groups.

Disclosure: Was this work supported by industry? No.

A-13
Maternal micronutrient deficiency (Cu or Zn or vit E) alters the expression of offspring insulin signaling pathway and predispose to insulin resistance and hypertension in later life

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Background: Epidemiological evidence suggests that some adult disorders like insulin resistance syndrome, hypertension and disease associated with it originate in fetal life. Maternal under-nutrition is hypothesized to predispose the offspring to disease in adult life. The relevance of maternal macronutrient deficiency has been well studied but not that of micronutrients. We hypothesize that chronic maternal dietary mineral (Cu or Zn or Fe) or vitamin E restriction modulate the expression of offspring's insulin signaling pathway individually predisposes the offspring to insulin resistance syndrome and hypertension in their later life.
Objective: To assess the effect of maternal dietary micronutrient (Cu or Zn or Vitamin E) restriction on insulin signaling and blood pressure in offspring.
Methods: Female weaning Swiss albino mice received a control diet (based on the American Institute of Nutrition AIN-93G diet) ($n=20$) or a 50% vitamin E-restricted diet ($n=20$) or 50% zinc-restricted diet ($n=20$) or 50% copper-

restricted diet ($n=20$) or 50% of iron-restricted diet ($n=20$) and they were provided with deionized water for 12 weeks and mated with control males. Pups born to the dams on the restricted diet were weaned on to the respective restricted diet. At the end of 20 weeks of feeding of the offspring, IPGTT was performed in the offspring. Adipose tissues were collected from offspring followed by tyrosine kinase activity; glucose utilization and turn over were measured. Systolic blood pressure was determined at 20-week-old offspring by tail cuff plethysmography using an IITC model-229 blood pressure monitor.
Molecular analysis: Adipose samples were used for Western blot analysis for expression profiles of various insulin signaling molecules in both control and micronutrient deficiency groups.
Results: Pregnant micronutrient-restricted dams had a higher abortion rate. Body weight and crown rump length were significantly ($p<0.001$) reduced in offspring, from dams fed micronutrient diet compared with control group. The micronutrient-restricted (Cu or Zn or Vit E) mice had significantly impaired glucose tolerance compared with control group. Glucose intolerance in association with hyperinsulinemia and increased systolic blood pressure suggests the presence of insulin resistance and hypertension in all the offspring of micronutrient-restricted groups. Western analysis of insulin signaling molecules were down-regulated in all the restricted diet fed groups.
Conclusion: It has been suggested that chronic maternal micronutrient deficiency alters insulin signaling pathways and predispose the offspring to IR and hypertension in later life.

Disclosure: Was this work supported by industry? No.

A-14
IUGR is associated with loss of stress kinase sensing and dissociation of AKT-MTOR signaling in liver and skeletal muscle

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In liver and skeletal muscle, signaling through PI 3-kinase to mTOR, along with AMP-kinase are involved in sensing and integrating signals from nutrient and growth factor availability to coordinate cell growth and metabolism. Here we identify the compensatory mechanisms associated with combined chronic adaptation to nutrient, oxygen, and hormone deficiency in an ovine model of fetal growth restriction (FGR). 7 control (C) and 12 placental insufficiency-induced FGR animals were studied. FGR animals were 44% of C fetal weights at term and had reduced arterial PO_2 (FGR: 12.02 ± 0.75 vs. $C18.97 \pm 0.63$ mm Hg). Circulating glucose, insulin, and IGF-I were all significantly reduced in FGR. Hepatic glycogen levels were paradoxically elevated (22%) in FGR, along with levels of the insulin receptor (IR), while GSK3β (a negative regulator of insulin signaling) was reduced, suggesting upregulation of proximal pathway for glycogen deposition. FGR livers had no change in mTOR, but a striking two-fold increase in 4EBP-1 and 45% decreased eIF4E suggesting a unique mechanism limiting translation. In muscle, upregulation of the IR and decreased p85α subunit (known to improve PI3-kinase activity) were observed; however, Akt-2 and mTOR were significantly downregulated, suggesting tissue-specific adaptation. No activation of AMPK or ER stress kinases was seen in liver or muscle. Both organs showed reduced phosphorylated eIF2-α suggesting loss of stress kinase sensing in FGR. These data suggest that FGR results in novel tissue-specific mechanisms for down-regulating protein translation. The unexpected loss of stress kinase signaling suggests this adaptation could compromise fuel sensing leading to future susceptibility to T2DM.

Disclosure: Was this work supported by industry? No.

A-15
Increased matrix metalloproteinase activity in endothelial cells exposed to umbilical cord plasma from high birth weight newborns

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Objective: Large for gestational age infants have increased risk of developing cardiovascular disease and metabolic syndrome later in life. The vascular endothelium is a pathogenetic target in many cardiovascular disorders. Here we asked if umbilical cord plasma of high birth weight (HBW, >4 kg) infants could modulate functional properties of human umbilical vein endothelial cells (HUVEC) compared with plasma from normal birth weight (NBW, 3.1—3.6 kg) infants.
Methods: Endothelial expression and release of matrix metalloproteinases (MMP) were used as indicators of endothelial function. HUVECs were exposed for 48 h to 20% venous cord plasma from uncomplicated term pregnancies with HBW and NBW fetuses.
Results: The MMP-activity in supernatants of HUVECs exposed to HBW-plasma was three times higher ($p<0.05$) than that obtained with NBW-plasma. MMP-9, but not MMP-2, protein concentration and mRNA expression were enhanced ($p<0.05$) in HBW. With specific blockers the increased MMP-activity and mRNA-MMP-9 could both be inhibited by about 70%. Cord lipid and insulin concentrations were similar ($p>0.05$) among the two groups. We could not detect significant differences between the two groups in concentrations of proinflammatory cytokines or specific tissue inhibitors of MMP in plasma or HUVEC supernatants.
Conclusion: Cord plasma from HBW infants induced more MMP-9 activity in HUVECs compared with cord plasma from NBW infants. Although not identified, cord plasma of HBW infants may contain factors that induce or activate endothelial cell MMP activity. These findings suggest an association between fetal nutritional conditions and endothelial cells functions.

Disclosure: Was this work supported by industry? No.

A-16

Early gestation undernutrition causes increased expression of myosin light chain kinase in adult sheep coronary arteries

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Objectives: We have previously shown that undernutrition in early gestation causes increased sensitivity of adult sheep coronary arteries to the vasoconstrictor acetylcholine [Khan et al. Ped Res 2005 58(5): 1043]. To elucidate the mechanisms underlying this effect, we examined expression of muscarinic M3 receptor and myosin-light-chain kinase (MLCK), an enzyme involved in the final common contractile pathway.
Methods: Welsh mountain ewes were fed 100% (CC, $n=7$) or 50% (UC, $n=6$) total nutrient requirements between 1 and 31 days gestation, and 100% thereafter. Postnatal offspring were fed *ad libitum*. Male offspring were sacrificed at 2.5 years and the distal anterior interventricular artery harvested. M3 receptor and MLCK mRNA levels were quantified using real-time PCR relative to 18S ribosomal RNA. Data are mean $\pm$ S.E.M. Intergroup comparison were made by ANOVA and Bonferroni *post hoc* tests.
Results: There was no difference between groups in M3 receptor mRNA levels (UC: 0.29 ± 0.06 vs. CC: 0.31 ± 0.06). MLCK mRNA levels were significantly elevated in UC compared to control offspring (UC: 1.12 ± 0.15 vs. CC: 0.68 ± 0.12; $p=0.044$).
Conclusions: These findings suggest that changes in MLCK rather than acetylcholine receptor may underlie the increased coronary artery sensitivity to acetylcholine observed previously in these early gestation nutrient-restricted offspring.
Supported by BHF, BUPA and HOPE.

Disclosure: Was this work supported by industry? No.

A-17

IUGR affects T-cell differentiation and thymic FoxP3 mRNA levels

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Objective: Intrauterine growth restriction (IUGR) increases postnatal infectious morbidities and glucocorticoid levels in both humans and rats. Since glucocorticoid administration selectively decreases naïve developing lymphocytes, we hypothesized that IUGR also results in a selective decrease of naïve, developing lymphocytes in the thymus at birth and alters the glucocorticoid responsive transcription factor FoxP3.
Design/methods: Bilateral uterine artery ligation and sham surgery were used to produce IUGR and control rats. Pups were harvested on day of life 0 and 21. Monoclonal antibodies and flow cytometry were used to compare T-cells (CD3+), T-helper (CD4+), T-cytotoxic (CD8+), and B-cells (CD45r+). FoxP3 was evaluated by real-time RT-PCR. IgA levels were analyzed with ELISA.
Results: Thymus weight and cell number were decreased in IUGR rats. IUGR also decreased the naïve, developing, double positive CD4+/CD8+ T-cells in the thymus. The percentages of mature, cytotoxic T-cells and B-cells in the thymus were increased in IUGR rats. Additionally, thymic FoxP3 mRNA levels and IgA serum levels were decreased in IUGR rats.
Conclusions: We conclude that IUGR disproportionately decreases undifferentiated T-cells in the postnatal thymus similar to glucocorticoid administration. IUGR also shifts the percentage of lymphocytes to a more mature, differentiated phenotype and results in decreased production of IgA. We speculate that elevated perinatal glucocorticoid levels lead to a persistent change in the postnatal lymphocyte population in the IUGR rat.

Disclosure: Was this work supported by industry? No.

A-18

IUGR alters the hepatic IGF1 histone code along the length the whole gene

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Objective: IUGR alters IGF1 levels in humans. In the rat, we have demonstrated that IUGR decreases postnatal IGF1 serum levels and affects hepatic IGF1 mRNA variants, particularly in males. IGF-1 is epigenetically regulated involving two promoters, alternative exon splicing of exons 2 and 5, and multiple termination sites. The objective of our study was to determine the histone code along the hepatic IGF1 gene at a resolution of 500—2000 bp in control (Con) and IUGR rats.
Methods: IUGR rats were generated by bilateral uterine artery ligation of pregnant e19 dams. Chromatin immunoprecipitation (ChIP) was performed with acetyl H3K4 and K9, dimethyl H3K4, and trimethyl H3K36 antibodies on control and IUGR D0 rat livers to characterize promoters P1 and P2, exon5, and alternative 3′UTRs.
Results: IUGR decreased D0 hepatic acetyl H3K9 at P2 ($p=0.016$), without affecting acetyl H3K9 or K14 at P1. This allowed us to use P1 as an intragenic control for dimethyl H3K4 and K36 ChIP. As expected, D0 dimethyl H3K4 increased in the P2 region of Con and IUGR IGF1, with IUGR decreasing IGF1 dimethyl H3K4 in males at exon 5 ($p=0.02$) and the proximal 3UTR ($p=0.008$). D0 trimethyl H3K36 increased within the transcribed region as expected, with IUGR having greater IGF1 trimethyl H3K36 at exon 5 ($p=0.04$).
Conclusion: Con and IUGR hepatic IGF-1 show unique patterns of covalent H3 modifications. Identification of these patterns along the whole gene and within the context of multiple modifications will define critical nucleosomes influenced by IUGR and allow us to study specific IGF1 chromatin modifying enzymes.

Disclosure: Was this work supported by industry? No.

A-19

Maternal food restriction affects postnatal hepatic IGF1 MRNA and chromatin structure in IUGR offspring

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Objective(s): Maternal food restriction (MFR) causes IUGR and leads to postnatal insulin resistance in the offspring. Hepatic IGF-1 may play a role in these morbidities. IGF-1 is epigenetically regulated involving two different promoters, alternative exon splicing of exons 2 and 5, and multiple transcription termination sites. Our objective was to determine if MFR affects IGF-1 hepatic mRNA levels and epigenetic characteristics in the offspring.
Material/method(s): Control dams ($n=5$) received ad libitum food, study dams (MFR, $n=5$) were 50% food-restricted from pregnancy days 10 to 21. All pups were nursed by dams fed ad libitum and were weaned to ad libitum feed. Male livers were collected at 8 weeks and 36 weeks. IGF-1 mRNA variant levels were quantified. Histone modifications were determined by Western blotting. Chromatin immunoprecipitation (ChIP) was subsequently performed, and associated levels of each IGF-1 species were measured by PCR.
Result(s): At 8 weeks of age, hepatic IGF-1 mRNA species were significantly decreased in the RP animals. MFR significantly increased hepatic dimethyl H3 lysine 4 (K4) levels on Western blots. Based on these results, ChIP was performed using dimethyl H3K4 antibodies. At 36 weeks of age, MFR significantly decreased dimethyl H3K4 at the IGF-1 P1 promoter ($p=0.006$) and exon 5 ($p=0.038$).
Conclusion(s): MFR decreases postnatal male hepatic IGF-1 mRNA levels and H3K4 dimethylation. We speculate the MFR decreases hepatic IGF-1 mRNA in the offspring by affecting IGF-1 epigenetic characteristics.

Disclosure: Was this work supported by industry? No.

A-20

P53 is necessary to signal the epigenetic changes that characterize the IUGR brain

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Objective: In humans, IUGR leads to postnatal changes in neurological function. In rats, IUGR changes epigenetic determinants of brain chromatin structure and gene expression. These changes persist postnatally. IUGR also increases brain p53 expression and activation. p53 belongs to complexes that modify epigenetic determinants of chromatin structure and affect gene expression. The objective of our study is to determine whether deletion of the p53 gene in mice prevents the changes we have previously observed in IUGR rat brains.
Methods: We produced Con and IUGR using sham surgery (CON) and bilateral uterine artery ligation in mice. Brains from newborn male mice were used in this study. Histone modifications were determined with Western blotting; total H3 or H4 were used as an internal control. Real-time RT-PCR was used to measure mRNA levels of genes known to be altered by IUGR and regulated by epigenetics.
Results: Similar to the rat, IUGR increased acetyl H3K9 and K14, as well as dimethyl H4R3, in p53+/+ brains vs. CON p53+/+ brains. IUGR also decreased dimethyl H3K9 in p53+/+ brains vs. CON p53+/+ brains. In p53−/− brains, IUGR did not increase acetyl H3K9, H3K14, and dimethyl H4R3. p53 genotype did not affect dimethyl H3K9 status. IUGR did affect 11beta HSD2 and IGF1 mRNA levels in the p53+/+ brains, and this effect was lost in the p53−/− mice.
Conclusion: p53 plays a role in triggering the epigenetic changes that occurs in the IUGR brain. This is important because p53 brain expression and activation respond to the environment induced by uteroplacental insufficiency that characterizes both the IUGR human and rat.

Disclosure: Was this work supported by industry? No.

A-21

Impact of embryo transfer and in vitro embryo culture during the periconceptional period on the expression of insulin-like growth factors and their receptors on fetal heart growth during late gestation in the sheep: large offspring syndrome revisited

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Objective: There have been no studies examining the differential impact of superovulation, artificial insemination (AI) and ET alone or combined with in vitro embryo culture (IVC) on fetal heart development in singleton and twin pregnancies, and have therefore tested the hypothesis that superovulation, AI and ET with or without IVC in the presence or absence of human serum (HS) differentially alters the growth of the fetal heart during development compared to naturally conceived controls and that these effects are different in singleton and twin pregnancies.
Methods: Embryos were collected donor ewes, which were subsequently transferred to one of three treatment groups: intermediate ewes until day 7 [ET group], an IVC of synthetic oviductal fluid either without [IVC+no serum (NS)], or with HS [IVCHS] until day 6. Embryos were then transferred to final recipient ewes. Naturally mated (NM) ewes were used as controls in this experiment. At 144/145 days gestation, ewes were killed. Real-time PCR was used to quantify mRNA levels of IGF-1 and IGF-2 present in fetal heart tissue.
Results: Relative heart weight was higher in the ET, IVCNS, and IVCHS groups compared to the NM group in singletons only ($p<0.05$). IGF-2 and IGF-2R expression was higher in

the IVCNS group. IGF-1R expression was significantly higher in singletons than twins in all groups.
Conclusions: In summary, ET and IVC appear to have differing effects on singleton and twin fetal heart development, possibly due to altered IGF signaling. Our results highlight the sensitivity of the developing embryo to an *ex vivo* environment and fetal number during the periconceptional period.

Disclosure: Was this work supported by industry? No.

A-22
Dietary phospholipid from cow's milk alleviates blood homocysteine level in pregnant rats with homocysteinemia

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Objective: Epidemiological studies suggest that homocysteinemia (HCM) in pregnancy is linked to periconceptional diseases and fetal growth retardation. It might be an important strategy to reduce blood level of homocysteine (HCy) in pregnant with HCM, and it could be crucial to activate betaine HCy methyl transferase (BHMT), an enzyme which remethylates HCy. Dietary phospholipid from cow's milk (MP) is rich in phosphatidyl choline. Since choline is a precursor of betaine, the substrate for BHMT, we hypothesized that MP might inhibit by BHMT increment of HCy level.
Materials/methods: Pregnant Sprague—Dawley rats were fed three experimental diets from gestational days (GD) 0.5 to GD20: choline-deficient diet (CD), by which the induction of HCM has been reported, CD supplemented with MP (CD+MP) or choline bitartrate (CD+C). Blood was taken from dams on GD10, and from dams and fetuses on GD20, and then analyzed on HCy and choline concentration. Liver was excised from dams and fetuses on GD20 to evaluate mRNA expression for HCy-related enzymes (now on evaluation).
Results: Plasma HCy level was clearly low in dams fed CD+MP and CD+C. The fetuses from dams fed CD+MP and CD+C had relatively low plasma HCy concentration compared with those from dams fed CD. Plasma choline level on GD20 was higher for CD+MP and CD+C dams than for CD dams. The fetuses from dams fed CD+MP had the highest plasma choline level amongst experimental groups.
Conclusion: Present study suggests that intake of MP is a favorable feeding regimen for reducing blood HCy level in pregnancy.

Disclosure: Was this work supported by industry? Yes: Meiji Dairies Corporation.
Do you act as a consultant, employee or shareholder with this industry? Yes.

A-23
Brain derived neurotrophic factor (BDNF) in the preterm and near-term ovine fetus and the effect of intermittent umbilical cord occlusion (UCO)

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Introduction: We sought to determine the regional distribution of BDNF as an important regulatory factor in the preterm and near-term ovine fetal brain and the effect of intermittent UCO.
Methods: Fetal sheep (control and UCO group at 0.75 and 0.90 of gestation) were studied over 4 days with UCO performed by inflation of an occluder cuff for 90 seconds every 30 min for 3 to 5 h each day. The fetal brains were perfusion-fixed and the distribution of BDNF immunoreactivity (IR) determined by immunohistochemistry using the Image-Pro Plus software to quantify the percent area stained.
Results: UCO caused a decline in arterial PO_2 (to 7 mm Hg) and pH (to 7.30), and rise in PCO_2 (to 61 mm Hg, all $p<0.01$) with a return to control values after the occluder release. BDNF IR decreased in the gray matter, thalamus, and hippocampus, but increased in the white matter ($p<0.01$) in control animals with advancing gestation in keeping with the developmental change from neurogenesis/gliogenesis to myelination over this period. UCOs resulted in ~ 50% decrease in BDNF IR for all brain regions in the preterm animals ($p<0.01$), whereas BDNF IR was increased in the hippocampus of the near-term animals ($p<0.05$).
Conclusion: Intermittent UCOs as studied have regional and gestational age dependent effects on the IR of BDNF within the ovine fetal brain with a global decrease in younger animals when protein turnover is higher, but a selective increase in the hippocampus of near-term animals consistent with heightened vulnerability for apoptotic injury, which might then impact on the brains development.

Disclosure: Was this work supported by industry? Yes: Canadian Institute of Health Research, Children's Health Research Institute, University of Western Ontario.
Do you act as a consultant, employee or shareholder with this industry? Yes.

A-24
Essential fatty acid deficiency in perinatal period alters hepatic gene expression in adult male mice

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Malnutrition in the perinatal period can program for altered metabolism in later life. We investigated the long-term effects of dietary essential fatty acid deficiency in the perinatal period on hepatic expression of genes involved in lipid metabolism.
Mice of C57BL/6 strain were given essential fatty acid deficient (EFAD) or control diet from gestational day 15 and throughout lactation. At 3 weeks of age, the pups were weaned to ordinary chow and body weight were recorded every week. Expression of peroxisome proliferator-activated

receptor gamma (PPARG) and PPARG coactivator 1 alpha (PGC1A) were analysed by quantitative real-time PCR in the livers from the offspring at 3 weeks of age and as 25-week-old adults.
At 3 weeks of age, the EFAD pups were smaller than the control pups, mean (S.D.) 4.0 g (0.5) vs. 9.1 g (0.9), and the transcript levels of PPARG and PGC1A were higher in EFAD livers than in controls, PPARG 6.85 (0.32) vs. 2.51 (0.43) and PGC1A 3.83 (0.42) vs. 1.15 (0.12). Hepatic expression of PGC1A continued to be higher in EFAD livers from adult male mice compared to controls, 0.91 (0.19) vs. 0.48 (0.06). In contrast, PPARG transcript levels were lower in EFAD than in controls, 0.57 (0.04) vs. 1.43 (0.29). The gene expression in female livers did not differ between the groups.
Essential fatty acid deficiency during perinatal period resulted in persistent changes in adult hepatic gene expression of PPARgamma and PGC1alpha in male mice, but no differences were seen in the female mice.

Disclosure: Was this work supported by industry? No.

A-25
Lipid metabolism is regulated by the liver X receptor in the fetal mouse liver

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Objective: The liver X receptors (LXRα/β) are vitally important in regulating cholesterol and fatty acid metabolism in the adult. We speculate that manipulation of LXR in early development may influence lipid metabolism during adulthood. Aim of this first study was to elucidate the role of LXR in the regulation of lipid homeostasis *in utero* in mice.
Materials/methods: Pregnant C57BL/6 mice were fed a diet containing the synthetic LXR agonist T0901317 from day 10.5 pc on. Gene expression and lipid levels were measured in fetuses and dams at various time points.
Results: *Lxra* is expressed at ~ 40% of the level found in liver of adult mice, whereas expression of *Lxrb* is 2.5—3 times higher than in adults. In fetal mouse liver, expression of LXR target genes is low. Upon administration of T0901317 to the diet of the dam, Lxr target genes are activated in the fetal liver: expression of the cholesterol transporter *Abcg5* is 45 times induced whereas the lipogenic genes *Srebp1c* and *Fas* are increased five-fold and two-fold, respectively. Consequently, triglyceride concentrations in fetal liver and plasma are raised up to 2.7- and 1.5-fold, respectively, after 1 week of treatment. Cholesterol levels in plasma are doubled upon treatment.
Conclusion: Lxr is functionally active in fetal liver when stimulated with the synthetic agonist T0901317. Our approach provides a means for experimentally interfering with lipid homeostasis in the fetus and may give more insight in the long-term effect of dysregulated lipid metabolism during development.
Supported by the Dutch Heart Foundation (2004T048).

Disclosure: Was this work supported by industry? No.

A-26
In utero undernutrition reduces newborn offspring renal microvascular density and endothelial nitric oxide synthase (ENOS)

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Objective: Maternally food-restricted (MFR) offspring develop hypertension as adults. We have previously reported reduced number of mesenteric microvessels and reduced glomerular number as potential etiologies of hypertension. We hypothesized that reduced angiogenesis is a global event in MFR offspring and a potential cause of the reduced nephrogenesis in these animals.
Materials/methods: Pregnant Sprague Dawley rats were food-restricted by 50% ($n=10$) or fed ad libitum ($n=10$) from e10 to term. Offspring were sacrificed on day 1 of life, and kidneys dissected and fixed in 4% paraformaldehyde. A specific eNOS antibody selectively stained microvessel endothelium in the vasa recta. The number of vessels and staining intensity (IOD) for eNOS were quantified (Image Pro Software) in 10 control and 10 MFR offspring obtained from different litters.
Results: Despite similar kidney weights in MFR and control offspring (0.070 ± 0.001 vs. 0.072 ± 0.002), there was a 20% reduction ($p=0.003$) in the number of microvessels in MFR offspring. Furthermore, the staining intensity (IOD) for eNOS was 1.6-fold lower ($p=0.004$) in the microvessels of MFR offspring as compared with controls.
Conclusions: Our findings demonstrate fewer renal microvessels and therefore potentially reduced blood flow to the kidney of MFR offspring. Decreased expression of eNOS indicates reduced vasodilatory influences and therefore another potential mechanism for reduced blood flow to the kidney of MFR offspring. These results suggest that reduced vascular supply may contribute to the reduction in glomerular number in these animals.

Disclosure: Was this work supported by industry? No.

A-27
Maternal undernutrition dysregulates fetal rat kidney growth factor signaling: mechanisms for decreased nephrogenesis and hypertension

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Objective: Maternal food restriction (MFR) causes reduced renal nephron number and systemic hypertension in offspring. TGFβ downregulation allows upregulation of growth factors EGF, IGF, FGF, GDNF, and PTN, which are necessary for nephrogenesis. We hypothesized that causing suppression of nephrogenesis in offspring of MFR dams is a result of altered growth factor signaling.
Materials/methods: Control dams received ad libitum food, whereas MFR dams were 50% food-restricted from pregnancy days 10 to 21. At birth, litter size was culled to eight offspring. All pups were nursed by control dams and weaned

at 3 weeks to ad libitum feed. Fetal kidneys at e16 were analyzed for TGFβ and TGFβ receptor, FGF, EGF, IGF, PTN mRNA expression by RT-PCR. Offspring kidneys at 8 weeks were analyzed for glomerular number.
Results: At e16, the kidneys of MFR fetuses had significantly higher expression of TGFβR1 (4.7 ± 0.4 vs. 2.4 ± 0.2 AU, $p < 0.01$), but no significant differences were seen in either TGFβR2, TGFβR3, or TGFβ 1 and 2 expression between the two groups. FGF8 (24-fold) and GDNF (10-fold) increased markedly, though there was no change in FGF 1 and 2, EGF, IGF, or PTN. At 8 weeks, there were 46% fewer glomeruli per kidney in the MFR offspring vs. controls.
Conclusions: MFR induces a very specific, selective increase in TGFβR1 expression of fetal kidney potentiating TGFß signaling and contributing to reduced nephron number. The increase in selective renal growth factors suggests a compensatory mechanism for nephrogenesis independent of TGFβ.

Disclosure: Was this work supported by industry? No.

A-28
High fat and protein diet during pregnancy and lactation alters hypothalamic adiponectin receptor gene expression in adult mouse offspring

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Objectives: The adipose-derived hormone adiponectin is involved in energy homeostasis, however its actions in the brain remain to be elucidated. Two adiponectin receptor subtypes have been identified (AdipoR1, AdipoR2). The aims of this study were to characterise AdipoR1 and AdipoR2 gene expression in the mouse hypothalamus, and determine whether exposure to maternal high fat-high protein diet (HFP) *in-utero* and during lactation modulates expression levels in adult offspring.
Methods: Female Balb/C mice were either fed a high fat-high protein diet (36.8% CHO, 32% lipid, 28% protein; $n = 9$) or standard chow (68.8% CHO, 10% lipid, 18% protein; $n = 6$) 6 weeks prior to conception through to pregnancy and lactation. Weaned offspring were fed standard chow until adulthood. Offspring were killed at 8 weeks old and hypothalamic brain blocks collected and analyze for changes in gene transcript levels by MT-qcPCR.
Results: In all offspring, hypothalamic AdipoR1 mRNA levels were 4-fold higher vs. AdipoR2 ($p < 0.001$). Moreover, AdipoR1 and AdipoR2 levels in males were 71% and 148% higher respectively vs. females ($p < 0.001$). In HFP males, but not in females, there was a 20% reduction ($p < 0.05$) in AdipoR2 levels vs. the chow group. No changes were observed in AdipoR1 expression.
Conclusions: Our results show that adiponectin receptors are differentially expressed in the hypothalamus according to receptor subtype and sex of the animal. We also found sex-specific effects of maternal HFP diet on receptor expression. It remains to be determined whether these receptors in the hypothalamus are involved in regulating energy balance.
Supported by MRC, BHF, and BBSRC.

Disclosure: Was this work supported by industry? No.

A-29
Effects of insulin on the expression and activity of system y^+L-mediated L-arginine transport in human umbilical vein endothelium

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L-Arginine transport is mainly mediated by systems y^+L and y^+/CATs in human umbilical vein endothelial cells (HUVEC). Insulin increases system y^+/CATs activity in this cell type, but the effect of this hormone on system y^+L has not been studied.
Objectives: We determined whether insulin regulates mRNA levels for *SLC3A2/4F2hc*, *SLC7A7/4F2-lc2*, and *SLC7A6/4F2-lc3* genes and transport activity of system y^+L in HUVEC.
Methods: L-[^{3}H]Arginine transport (0.6—20 μM, 2 μCi/ml, 1 min, 37 °C) was measured in absence or presence of insulin (0.1 nM, 8 h), wortmanin (30 nM, PI3-k inhibitor), Na^+, *N*-ethylmaleimide (200 μM, system y^+ inhibitor), or L-leucine (100 μM). mRNA levels of *SLC3A2/4F2hc*, *SLC7A7/4F2-lc2*, and *SLC7A6/4F2-lc3* genes for system y^+L were analyzed by RT-PCR.
Results: Insulin increased (~ 80%) the transport capacity (V_{max}/K_m) for system y^+L, mainly by modifying the maximal velocity (V_{max}). Insulin also increased *SLC3A2/4F2hc* (~ 60%) expression. Wortmanin reduced insulin effects on L-arginine transport and *SLC3A2/4F2hc* expression. However, insulin reduced *SLC7A7/4F2-lc2* (~ 20%) and *SLC7A6/4F2-lc3* (~ 25%) mRNA levels. Wortmanin returned to basal levels the expression of *SLC7A7/4F2-lc2*, but had no effect on the expression of *SLC7A6/4F2-lc3*.
Conclusions: Our results suggest that insulin increases system y^+L activity possibly through transcriptional mechanisms mainly by increasing the *SLC3A2/4F2hc* expression via PI3-k signalling pathway in human umbilical vein endothelium.
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Disclosure: Was this work supported by industry? No.

A-30
Insulin restores D-glucose reduced adenosine transport in human umbilical vein endothelium

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Adenosine transport is mediated by equilibrative nucleoside transporters 1 (hENT1) and hENT2 in human umbilical vein endothelial cells (HUVEC). High D-glucose (25 mM) reduces adenosine transport via hENT1 in this cell type; however, nothing is known regarding insulin effect on adenosine transport in endothelium.
Objective: We have studied whether insulin was able to modulate adenosine transport via hENT1 and/or hENT2 in

primary cultures of HUVEC exposed to elevated extracellular D-glucose.
Methods: HUVEC were isolated from normal pregnancies and cultured for 24 h in medium 199 containing 5 or 25 mM D-glucose, in absence or presence of 0.1 nM insulin (for the last 8 h of the 24 h incubation period). Adenosine transport was determined in absence or presence of nitrobenzylthioinosine (NBTI, 1 μM, inhibitor of ENT1) and/or hypoxanthine (2 mM, inhibitor of ENT2). Citrulline assay was used to determine endothelial nitric oxide synthase (eNOS) activity. hENT1, hENT2, and eNOS protein abundance was detected by Western blot, and mRNA levels quantitated by real-time RT-PCR.
Results: Insulin did not alter high D-glucose effect on hENT1 mediated transport and expression, but increased hENT2 mediated transport and expression. Changes in hENT2 transport activity were reflected as a significant increase of total adenosine transport (i.e. hENT1 + hENT2 mediated). In addition, insulin increased hENT2 expression, but reduced hENT1 expression. Insulin also increased synthesis of nitric oxide (NO) and expression of endothelial NO synthase (eNOS).
Conclusion: Insulin restores high D-glucose inhibited adenosine uptake in HUVEC. This hormone could be acting as a protective mechanism involved in the maintenance of a normal extracellular level of adenosine in the fetal circulation limiting its vascular effects.
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Disclosure: Was this work supported by industry? No.

A-31
Growth trajectory and regression to the mean in the foetal origins hypothesis

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Objective: A number of recent publications have found that small birth size in conjunction with rapid growth yields a greater risk of developing adult chronic diseases. Several recent articles show that people who develop diseases such as coronary heart disease and diabetes experience a different growth trajectory from those who do not. This study critically evaluates this evidence.
Materials/methods: Using the principles by which Sir Francis Galton discovered the phenomenon of regression to the mean, we explain why evidence supporting the hypothesis of different growth trajectories is not conclusive.
Results: In the presence of an inverse association of later disease with body size at birth, and a positive association with body size later in childhood, the growth trajectory in individuals who experience later disease must inevitably exhibit centile crossing. Therefore, presentation of growth trajectories according to health outcomes does not provide evidence supporting associations of catch-up growth as a risk factor for disease in later life. Seemingly different growth trajectories represent only a series of cross-sectional associations between health outcomes and body size at each age throughout the life course. Data from the previous DOHAD meeting is used to illustrate this and the correct interpretation is discussed.
Conclusion: The interpretation of growth trajectories in the foetal origins hypothesis needs to consider the impacts of regression to the mean; evidence purporting to support the catch-up growth hypothesis is still pending.

Disclosure: Was this work supported by industry? No.

A-32
Fetal programming of plasma insulin and skeletal muscle expression of GLUT4 and UCP3

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We recently demonstrated that postnatal dietary omega-3 fatty acids can attenuate glucocorticoid-induced programming of hypertension and hyperleptinemia in the rat. The present study determined programmed changes in plasma insulin, serum glucose, and skeletal muscle GLUT4 and UCP3 expression. We also determined whether any programmed changes were altered by a postnatal diet rich in omega-3 fatty acids.
Dexamethasone was administered to pregnant rats from day 13 to term. The offspring of treated and control mothers were cross-fostered to mothers on either a standard or high omega-3 diet, and remained on these diets post-weaning. In 6-month-old rats, insulin was measured by radioimmunoassay, glucose by hexokinase assay and mRNA expression of GLUT4 and UCP3 were assessed by quantitative RT-PCR.
Hyperinsulinemia was evident in offspring exposed to dexamethasone *in utero* and raised on a standard diet, but this effect was completely blocked by a high omega-3 diet from birth. Serum glucose was unaffected by either prenatal treatment or postnatal diet. In skeletal muscle, GLUT4 expression was elevated by up to 20-fold in dexamethasone-exposed offspring but this effect was not altered by postnatal diet. Fetal glucocorticoid excess also decreased UCP3 expression, but again was not affected by diet.
These results demonstrate for the first time that a postnatal diet high in omega-3 fatty acids can attenuate glucocorticoid-induced programmed hyperinsulinemia. Additionally, skeletal muscle in the programmed offspring exhibits aberrant expression of genes involved in glycaemic control, but this effect is not altered by postnatal diet.

Disclosure: Was this work supported by industry? No.

B. COHORT STUDIES

B-01
Associations of early growth and catch-up with blood pressure in British White European and South Asian origin babies: the Manchester Children's Heart and Growth Study

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Specific indices of early postnatal growth may be markers for the development of adult cardiovascular disorders, whose risk differs between ethnic groups.
Objective: To examine size at birth, catch up growth and their associations with blood pressure (BP), in British born South Asian (SA) and White European (WE) infants in the first 2 years of life.
Subjects/methods: Women were recruited in pregnancy, with detailed measurements taken at 28 weeks. Their healthy infants born at term were followed up with similarly standardized measures of weight (W), length (L), head circumference (HC), and BP analyzed using multilevel modeling and correlations. 367 babies (249 WE, 118 SA) were measured.
Results: Over the 24 months, SA weighed less than WE babies by 0.38 SDS (95% CI 0.57, 0.18) and had a lower BMI SDS by 0.39 (0.2, 0.59). They were shorter by 0.22 SDS (0.43, 0.01) for L-SDS and had 0.49-SDS (0.70, 0.28) smaller HCSDS. No significant difference in mean (SD) systolic BP (SBP) between the two ethnicity except at 24 months.
Catch-up growth for W-SDS and BMI-SDS was greater over the first 3 months in SA than WE (ΔW-SDS 0.1 (1.0) vs. −0.28 (0.9), $p=0.005$).
SBP at 24 months correlated positively with change in W, L and HC at 24 months ($r=0.5$, $p=0.000$, $r=0.3$, $p=0.006$, $r=0.3$, $p=0.018$).
Conclusions: Significant ethnic differences in growth and catch-up are evident in this cohort, particularly over the first 3 months of life but not 1 year BP. These differential patterns may affect later cardiovascular and metabolic risk.

Disclosure: Was this work supported by industry? Yes: Diabetes UK.
Do you act as a consultant, employee or shareholder with this industry? No.

B-02
Maternal and early life determinants of serum adiponectin from birth to 4 years in a multi-ethnic population: the Manchester Children's Cardiovascular Health Study

Bansal, N[1]; McElduff, P[2]; Gemmell, I[1]; Vyas, A[1]; Pemberton, P[1]; Charlton-Menys, V[1]; Durrington, PN[1]; Clayton, PE[1]; Cruickshank, JK[1]

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Objective: To investigate the maternal and early life determinants of serum adiponectin in 0—4-year-old children of White European (WE) and South Asian (SA) origin.
Materials and methods: 196 children (261 observations, 65 longitudinal) were studied, both cross-sectionally and longitudinally, between 0 and 4 years of age (at birth, 3, 6, 12, 24, 36 and 48 months) for measures of diet, anthropometry (height, weight, skinfold thickness; suprailiac, triceps, and subscapular), BP, serum lipids (TC, LDL-C, HDL-C, triglycerides, and NEFA), and adiponectin. Maternal adiponectin and anthropometric characteristics were assessed during pregnancy at 28 weeks gestation. Data from all postnatal timepoints were analysed together with maternal data using an age adjusted mixed longitudinal model.
Results: At 28 weeks gestation, maternal adiponectin and HDL-C was lower in SA compared to WE ($p=0.07$ and $p<0.001$ respectively). In an age adjusted analysis including child weight, skinfold thickness, head circumference, HDL-C, ethnicity, sex and maternal adiponectin, maternal adiponectin ($\beta=0.7$, 95% CI 0.5 to 0.9, $p<0.001$), child weight ($\beta=0.3$, 95% CI 0.05 to 0.6, $p=0.02$), HDL-C ($\beta=1.3$, 95% CI 0.5 to 2.2, $p=0.003$), length ($\beta=-0.2$, 95% CI -0.4 to -0.04, $p=0.02$), head circumference (-0.4, 95% CI -0.7 to -0.1, $p=0.004$), ethnicity (being WE) ($\beta=1.6$, 95% CI 0.5 to 2.8, $p=0.006$), and sex (being female) ($\beta=-0.8$, 95% CI -1.5 to -0.2, $p=0.02$) were significant predictors of child adiponectin.
Conclusion: Ethnic differences in HDL-C are not present in early life; however, adiponectin levels are significantly lower in SA children compared to those of WE origin.

Disclosure: Was this work supported by industry? No.

B-03
Maternal and early life determinants of serum lipids in infancy and early childhood: the Manchester Children's Cardiovascular Health Study

Bansal, N[1]; McElduff, P[2]; Gemmell, I[1]; Charlton-Menys, V[1]; Vyas, A[1]; Oldroyd, J[3]; Clayton, PE[1]; Durrington, PN[1]; Cruickshank, JK[1]

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Introduction: Adverse lipid profiles track from child to adulthood. Direct and indirect evidence suggest that maternal cholesterol may contribute to the foetal circulation during gestation. Little is known about the maternal determinants of serum lipids in young children.
Objective: To investigate the early life and maternal determinants of serum lipids in 0—4-year-old children.
Materials and methods: As part of our ongoing mixed longitudinal prospective study, 196 children were studied, both cross-sectionally and longitudinally, between 0—4 years of age (at birth, 3, 6, 12, 24, 36, and 48 months) for measures of diet, anthropometry (height, weight, skinfold thickness, suprailiac, triceps, and subscapular), BP, and serum lipids (TC, LDL-C, HDL-C, triglycerides, and NEFA). Maternal lipids and anthropometric characteristics were assessed at 28 weeks gestation. Data from all postnatal timepoints were analysed together with maternal data using an age adjusted mixed longitudinal model.
Results: Maternal TC ($\beta=0.1$, 95% CI 0.04 to 0.2, $p=0.007$) and skinfold thickness ($\beta=0.06$, 95% CI 0.02 to 0.09, $p=0.005$) were significant predictors of child TC. Maternal LDL-C ($\beta=0.1$, 95% CI 0.002 to 0.2, $p=0.045$) and HDL-C ($\beta=0.2$, 95% CI 0.09 to 0.4, $p=0.002$) were the only significant predictors of the respective lipids in their children.
Conclusion: Maternal TC is an independent predictor of child TC, mainly attributable to the strong positive relationship between maternal—child HDL-C.

Disclosure: Was this work supported by industry? No.

B-04
Influence of birth weight on bone size and mineral content in young Gambian adults

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We investigated whether birth weight (BWt) and weight in early adulthood (Wt) influence bone size and BMC in a cohort of 54 nulliparous women and 76 men, aged 17—21 years, from the rural African village of Keneba, The Gambia.
DXA (Lunar-DPX) measurements were carried out at forearm, hip, spine, and whole body. BWt was obtained from clinic records (mean ± S.D.; BWt males 3.04 ± 0.37 kg, BWt females 2.92 ± 0.37 kg) and current Wt measured (mean ± S.D.; Wt males 53.2 ± 6.70 kg, Wt females 54.3 ± 6.20 kg). Statistical analysis combined univariate and multiple regression analysis of BWt adjusted for Wt and Wt adjusted for BWt; BMC was analysed unadjusted and adjusted for bone area (BA). Results were considered significant at $p<0.05$.
In both univariate and multivariate analyses, BWt was a positive predictor of bone size (males and females: radial shaft width, femoral neck width; females only: total hip area, vertebral height, whole body area), but did not predict BA-adjusted BMC. Wt positively predicted BMC at all sites and BA-adjusted BMC (except at trochanter in males). Wt also predicted bone size at the spine and at the hip in males. Wt did not significantly predict bone size at the forearm or hip in females.
These results agree with previous pQCT work on this cohort, showing that BWt is an independent predictor of skeletal size in early adulthood, but not of BMC. Wt mainly predicted BMC. Therefore, BWt and Wt appear to exert separate and different influences on the skeleton in early adulthood, which may have implications for long-term bone health.

Disclosure: Was this work supported by industry? No.

B-05
Birth weight at term and respiratory symptoms in young children: interaction with parental smoking—the Piama birth cohort

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Objective: Perinatal factors influence the risk of respiratory disease in early life. However, the relation between birth weight and respiratory symptoms and/or asthma in children born at term remains unclear. Our objective was to estimate the risk of respiratory symptoms and asthma in relation to birth weight in term born children in the first 7 years.
Methods: In a prospective birth-cohort study, 3628 children with a gestational age ≥ 37 weeks were followed for 7 years. Parental questionnaires were used to assess respiratory health every year. Associations of birth weight with respiratory symptoms (wheezing, coughing, respiratory infections) and a doctor's diagnosis of asthma were assessed in a repeated-event analysis.
Results: A lower birth weight was associated with more respiratory symptoms (odds ratio [OR] per kilo decrease in birth weight: 1.21, 95% confidence interval [CI]: 1.09—1.34). The association appeared to increase from age 1 to 5, but decreased thereafter and was no longer significant at the age of 7. The effect of birth weight on respiratory symptoms was significantly greater among children exposed to tobacco smoke in their home (OR at 5: 1.52, 95% CI: 1.23—1.87) than among non-exposed children (OR at 5: 1.21, 95% CI: 1.02—1.44). No significant relation was found between birth weight and asthma diagnosis (OR: 1.06, 95% CI: 0.82—1.37).
Conclusions: A low birth weight in children born at term is associated with a transient increased risk of respiratory symptoms. This effect is enhanced by environmental tobacco smoke exposure.

Disclosure: Was this work supported by industry? No.

B-06
Growth in early life and reproductive disorders in young women

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Objective: Adult body size and composition are positively associated with metabolic disorders and reproductive functioning in women. However, findings specific to growth in early life and symptoms of polycystic ovary syndrome (PCOS) are inconsistent. We aimed to examine the relationship between specific symptoms of PCOS in adulthood and birth phenotype within a population derived birth cohort.
Materials/methods: We defined a birth cohort of all female babies born 1973—1975 who survived to discharge from The Queen Elizabeth Hospital, a major hospital in Adelaide, South Australia. We have traced 86% of the cohort to date, of whom 62% live in Adelaide. Of this group, 55% ($n=691$) have been interviewed and had a physical assessment by trained research staff. Risk ratios for reproductive disorders were calculated using generalised estimating equations.
Results: Birth anthropometry was very similar to population norms. The prevalence of an existing diagnosis of PCOS at interview was 5.5%. A pre-existing diagnosis of PCOS at interview was negatively associated with birth weight, chest, head, and abdomen circumferences ($p<0.05$). In contrast, the syndrome-specific symptom of hirsuitism (Ferriman-Gallway score of 8+) was positively associated

to birth weight, length, chest, and abdomen circumference ($p<0.09$), while menstrual irregularity was positively associated with placental size ($p<0.05$).
Conclusion: A diagnosis of PCOS by age 30 is related to reduced growth to birth, unlike the symptoms of hirsuitism and menstrual irregularity, which relate positively to size at birth, implying differing aetiological pathways.

Disclosure: Was this work supported by industry? No.

B-07
Maternal smoking and the risk of respiratory tract infections in infancy. The Generation R Study

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Objectives: The aim of this study was to examine the associations of prenatal and postnatal maternal smoking with respiratory tract infections in their infants.
Methods: This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. Data were assessed by questionnaires. Maternal smoking was registered in early and late pregnancy and 6 months postnatally. Doctor-diagnosed upper and lower respiratory tract infections of their infants were recorded at the age of 6 months. The present analyses were based on the first 2671 infants of whom complete data were available.
Results: Maternal smoking in late pregnancy and postnatal maternal smoking were both associated with an increased risk of lower respiratory tract infections in infants at the age of 6 months (OR (95% confidence interval) 2.42 (1.25, 4.69) and 2.29 (1.30, 4.04)). The effect of maternal smoking in late pregnancy was smaller after adjusting for postnatal maternal smoking and other confounders (OR 1.57 (0.61, 4.00)). The effect of postnatal maternal smoking did not materially change after adjusting for prenatal maternal smoking and other confounders (OR 2.08 (1.00, 4.30)). No associations of maternal smoking in early pregnancy with lower respiratory tract infections or maternal smoking and upper respiratory tract infections were found.
Conclusions: Postnatal maternal smoking increases the risk of lower respiratory tract infections in infants at the age of 6 months. The association of maternal smoking late in pregnancy with lower respiratory tract infections in infants was largely explained by postnatal maternal smoking.

Disclosure: Was this work supported by industry? No.

B-08
Maternal essential fatty acid status in pregnancy and neonatal birth weight: first results of the Amsterdam born children and their development birth cohort

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Objective: To investigate the association between maternal essential fatty acid (FA) status in early pregnancy and birth weight in a large-scale prospective cohort study.
Methods: Pregnant women donated blood at their first antenatal screening (12th pregnancy week) for fatty acid measurement in plasma phopholipids and filled out a questionnaire on lifestyle, sociodemographics, and psychosocial conditions. Using univariate and multivariate linear regression analysis, associations between maternal linoleic (C18:2n-6, LA) arachidonic (C20:4n-6, AA), α-linolenic (C18:3n-3, ALA), eicosapentaenoic (C20:5n-3, EPA), and docosahexaenoic (C22:6n-3, DHA) acid concentrations and birth weight (term deliveries, $n=3725$) were studied.
Results: All maternal n-3 FAs were positively associated with birth weight: 1 S.D. increase in relative concentration of ALA, EPA, and DHA corresponded to birth weight increases of 23, 23, and 24 g respectively ($p<0.01$). In contrast, negative associations were found for n-6 FAs: 1 S.D. increase in relative concentration of LA and AA corresponded to birth weight decreases of -28 and -23 g respectively ($p<0.01$). After adjustment for gestational age, infant's sexes, and maternal characteristics (parity, age, height, BMI, smoking, alcohol consumption, ethnicity, and educational level) only the positive association for DHA (+15 g with 1 S.D. increase, $p<0.05$) and the negative association for AA (-20 g with 1 S.D. increase, $p<0.01$) remained significant.
Conclusions: Fetal growth is influenced both by n-3 (DHA) and n-6 fatty acids (AA), however in opposite ways. Although further research should elucidate these presumably competitive roles, it seems prudent to encourage pregnant women to increase DHA intake (fatty fish) while minimizing AA intake (meat).

Disclosure: Was this work supported by industry? No.

B-09
Effects of low birth weight in 8- to 13-year-old children: implications in endothelial function and uric acid levels

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Objectives: Low birth weight has been associated with an increased incidence of adult cardiovascular disease. Endo-

thelial dysfunction and high levels of serum uric acid are associated with hypertension and endothelial dysfunction. We have determined whether uric acid is related to blood pressure and vascular function in children with low birth weight.
Materials/methods: We evaluated the vascular function by high-resolution ultrasound, blood pressure and uric acid levels in 78 children (35 girls, 43 boys, aged 8 to 13 years).
Results: Increasing levels of uric acid and systolic blood pressure were observed in children with low birth weight. Birth weight was inversely associated with both systolic blood pressure and uric acid. On the other hand, uric acid levels were directly correlated with systolic blood pressure in children of the entire cohort. Low birth weight was associated with reduced flow-mediated dilation ($r = 0.427$, $p < 0.001$). Since the children with low birth weight had elevated uric acid as well as higher systolic blood pressure levels, we evaluated the correlation between these variables. In the low birth weight group, multiple regression analysis revealed that uric acid ($\beta = -2.886$, S.E. = 1.393, $p = 0.040$) had a graded inverse relationship with flow-mediated dilation, which was not affected in a model adjusting for race and gender.
Conclusion: We conclude that children with a history of low birth weight show impaired endothelial function and increased blood pressure and uric acid levels. These findings may be early expressions of vascular compromise, contributing to susceptibility to disease in adult life.

Disclosure: Was this work supported by industry? No.

B-10
Maternal anthropometrics in pregnancy and left ventricular mass in infancy. the Generation R Study

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Objectives: Gestational diabetes is characterized by an increase in weight during pregnancy and is associated with larger left ventricular size in infancy. The aim of this study was to examine the association of maternal weight in pregnancy with left ventricular mass in infancy in a population-based cohort.
Methods: This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until adulthood. Maternal anthropometrics were obtained in early (gestational age < 18 weeks), mid- (gestational age 18—25), and late (gestational age > 25 weeks) pregnancy. Echocardiographic follow-up measurements were performed in a subgroup of 791 infants aged 6 weeks and 6 months.
Results: No associations were found of weight or body mass index measured in early, mid- and late pregnancy with left ventricular mass at the age of 6 weeks and 6 months. Maternal weight gain and increase in body mass index during pregnancy were positively associated with postnatal left ventricular mass at 6 months of age (0.08 (95% CI: 0.02, 0.15) g/kg increase and 0.24 (95% CI: 0.05, 0.43) g/kg/m^2 increase, respectively). These associations did not materially change after adjustment for maternal weight and height at intake, and gender, age, weight, and length of the child.
Conclusion: Increase in maternal weight and body mass index during pregnancy are associated with larger left ventricular mass at the age of 6 months. These findings suggest that maternal anthropometrics during pregnancy may have consequences for left ventricular mass in their children.

Disclosure: Was this work supported by industry? No.

B-11
Ethnic differences in birth weight and preterm birth: results of the Amsterdam Born Children and their Development study

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Objectives: To determine whether ethnic differences in birth weight and preterm birth exist, and to what extent these differences can be explained by relevant determinants.
Methods: As part of the prospective multi-ethnic Amsterdam Born Children and their Development (ABCD) study, differences in birth weight (≥ 37.0 weeks of gestation) and preterm birth between immigrant's and Dutch newborns were analyzed using respectively multivariate linear regression and multivariate logistic regression. Analyses were adjusted for physiological (gender, maternal height, weight, age, parity, hypertension, and vaginal problems) and environmental factors (education, marital status, smoking, alcohol use, depression, work stress). Only singleton deliveries were included ($n = 7494$).
Results: All immigrant groups had on average smaller babies than the Dutch group. After adjustment the Surinamese, Antillean, and Ghanaian newborns were smaller (*B* (S.E.): −97.7 (24.7), −114.1 (48.9), −133.4 (37.9)), the Turkish newborns were heavier (*B* (S.E.): 57.5 (27.7)), and the Moroccan newborns had a similar weight (*B* (S.E.): 8.9 (22.2)) compared to the Dutch newborns. The physiological factors were mainly responsible for reducing the differences in birth weight. The adjusted risk for preterm birth was higher in the Surinamese (OR: 1.8, 95% CI: 1.3—2.7), Antillean (OR: 1.9, 95% CI: 0.9—4.0), and Ghanaian (OR: 2.7, 95% CI: 1.6—4.7) groups compared to the Dutch group. The Turkish and Moroccan groups did not have a higher risk on preterm birth.
Conclusions: We conclude that birth weights and preterm births differ among ethnic groups, which is mainly explained by physiological determinants. The Surinamese, Antillean, and Ghanaian newborns are at higher risk for low birth weight and preterm birth.

Disclosure: Was this work supported by industry? No.

B-12
Early and late determinants of physical activity in adulthood

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Objective: Physical activity is a known health protective factor, benefiting metabolic and psychological aspects. Some studies state that physical activity can be programmed by early life events, although the environment also influences such variables. The objective of this study was to verify early and late determinants of physical activities in young adults.
Materials/methods: 2063 individuals from a cohort study born from June 1978 to May 1979 in Ribeirão Preto, Brazil, were studied at the age of 23/25 years. Logistic regression was performed using three models: (1) early model considering the variables birth weight, gestational age, ponderal index, and maternal socioeconomic, schooling, and smoking data collected shortly after birth; (2) late model considering participant's socioeconomic, schooling, and smoking data; (3) combined (early+late) model. Physical activity was evaluated according to the CELAFISCS and CDC proposal, considering two categories: active and sedentary.
Results: The rate of sedentary activity was 58.7%. In the early model, low birth weight (OR=2.86, 95% CI 1.61—5.09) and maternal schooling (OR=2.54, 95% CI 1.54—4.19) were risk factors for sedentary activity. The interruption of smoking (OR=0.47, 95% CI 0.27—0.80) had a late protective influence on sedentary activity. In the combined model, birth weight and interruption of smoking remained significant.
Conclusion: These findings contribute to the hypothesis that early life events in combination with environmental factors can significantly impact later behaviour.

Disclosure: Was this work supported by industry? No.

B-13
Body size at birth is associated with pessimistic life orientation at late adulthood: longitudinal study among women and men born at term in Helsinki 1934—1944

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Objective: Both prenatal factors and personality traits have been shown to be related to several physical and psychological health outcomes. However, few studies have examined the association between prenatal factors and adult health related personality traits, and even smaller amount among adults born at term. We studied whether gestational age and body size at birth predict pessimistic life orientations.
Materials/methods: The participants were 1374 Helsinki Birth Cohort members born at term in 1934—1944 and followed to age 59.7—70.7 years. Subjects completed the Life Orientation Test-Revised (LOT-R) on two separate occasions (mean time between measurements 1.88 years). The mean of the LOT-R scores was used. Data abstracted from birth records included gestational age, weight, length, and head circumference.
Results. After adjustments for sex and gestational age, lower birth weight was associated with higher pessimism (p for trend = 0.02). Further, there were two quadratic associations: those born shorter and taller, and those having shorter and longer head circumference at birth were more pessimistic than those in between (p's for trend < 0.003). Finally, participants belonging to the smallest group (weight ≤ 2.5 kg, length < 48 cm, or head circumference < 33 cm) at birth were significantly more pessimistic compared to the rest of the participants (p's < 0.02). Adjustment for childhood and adulthood socioeconomic status, age, and BMI had little effect on the results (p's < 0.04).
Conclusions: Body size at birth is associated with the health related personality, higher pessimism, in late adulthood even among those who have been born at term.

Disclosure: Was this work supported by industry? No.

B-14
Maternal pre-pregnancy weight and weight gain during pregnancy and newborn anthropometry in the EDEN Study (study of pre- and postnatal determinants of children growth and development)

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Objective: Early determinants of adiposity are of importance in the context of increasing prevalence of obesity i. We investigated the relationships between maternal anthropometry before and during pregnancy and newborn anthropometry.
Methods: The EDEN Study was proposed to all pregnant women visiting selected maternities before the 22nd week of gestation. Anthropometric measures were performed between 22 and 26 weeks of gestation on mothers and 3 days after delivery on mothers and babies. For the 731 first included women, newborn anthropometry was analyzed in relation with pre-pregnancy BMI (pBMI), mother's weight (mWG), and subcutaneous adiposity change in regressions adjusted for mother's age, centre, newborn gender, and gestational age.
Results: Obese women gained less weight than their peers during pregnancy (3.6 ± 0.7 vs. 9.5 ± 0.2 kg, $p = 0.0001$), and lost more subcutaneous fat during the last 3 months of pregnancy $p = 0.001$). Birth weight and newborn's subcutaneous skinfolds increased with pBMI (p-trends < 0.001). In a multivariate regression, anthropometry at birth was associated with pBMI (p-trends < 0.001) and with mWG, although more weakly ($p = 0.05$). Mothers who lost more subcutaneous adiposity during the last 3 months of pregnancy had fatter babies at birth independently of pBMI ($p = 0.05$).

Conclusion: The stronger relation of neonate anthropometry with pre-pregnancy BMI than with weight gain might be explained by the ability of fatter women to mobilise energy from fat store to sustain fetal growth during the last 3 months of pregnancy.

Disclosure: Was this work supported by industry? No.

B-15

Low lean-body-mass in young adults with very low birth weight

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Objective: We have previously described an association between very low birth weight (VLBW, <1.5 kg) and impaired glucose regulation. Our aim was to assess whether this association is explained by differences in body size and composition.
Materials/methods: At the age of 18 to 27 years, body composition was measured by dual-energy X-ray absorptiometry in 148 VLBW-born subjects (62 men) and 136 term-born controls (55 men) matched for age, gender, and birth hospital. Gestational ages were 29 weeks (S.D. 2.2 weeks) and 40 weeks (1.2 weeks), birth weights were 1.1 kg (0.2 kg) and 3.6 kg (0.47 kg), and birth weight S.D. scores were −1.3 (1.5) and 0.0 (1.0).
Results: VLBW-born adults were shorter (women by 5.3 cm, 95% CI 3.2 to 7.3 cm, men by 6.0 cm, 3.7 to 8.4 cm) and had lower lean-body-mass (women by 9.5%, 5.6 to 13.2%, men by 13.0%, 8.5 to 17.4%). Their height-adjusted lean-body-mass was lower as well (women 3.1% 0.0 to 6.1%, men 5.5%, 1.5 to 9.3%) but percent-body-fat was similar. Adjustments for percent-body-fat, lean-body-mass, and height did not explain the higher glucose and insulin concentrations in the VLBW group. Within the VLBW and control groups, height-adjusted lean-body-mass was not significantly affected by gestational age, birth weight, or birth weight SDS.
Conclusions: Men and women born with VLBW have lower lean-body-mass, even after allowance for their shorter height. This does not, however, explain the observed differences in glucose tolerance.

Disclosure: Was this work supported by industry? No.

B-16

Pre- and postnatal growth patterns: association with adolescent insulin-like growth factor-I (IGF-I) levels. The Copenhagen Follow-up Study of Fetal Growth (COFFEG)

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IGF-I is an important promoter of pre- and postnatal growth; thus, IGF-I levels are low in small for gestational age (SGA) newborns. We hypothesize that the IGF-I level is important for postnatal catch-up growth.
A prospective study in 1985–1987 ($N=594$) assessed third trimester fetal growth velocity (FGV) by ultrasonography, anthropometry at birth and 1 year of age. The 10 percentile defined SGA and intrauterine growth restriction (IUGR), respectively. From this birth cohort, 121 adolescents (52 males) participated in a follow-up study. Near-final height was measured, serum IGF-I determined by RIA, and parental height obtained by a questionnaire.
In this cohort birth weight (BW) SDS ($B=0.27$, $p=0.0001$), but not third trimester FGV ($B=0.15$, $p=0.48$), was significantly associated with adolescent height SDS (corrected for target height). In a multiple regression analysis adolescent IGF-I was inversely associated to BW SDS ($B=-0.40$, $p=0.025$), not associated to FGV ($B=0.15$, $p=0.72$), borderline associated to growth in first year of life ($B=-0.38$, $p=0.06$), and positively associated to adolescent height SDS (corrected for target height) ($B=0.47$, $p=0.015$). In the SGA group ($N=47$) individuals with catch-up growth (delta height SDS > 0.67 from birth to adolescence) had significantly higher IGF-I (−0.38 vs. −1.37 SDS, $p=0.03$) than those without catch-up growth with similar target height.
In conclusion, BW but not third trimester FGV has an independent impact on the postnatal growth pattern. Adolescent IGF-I levels were inversely correlated with BW SDS. SGA children with catch-up growth had significantly higher IGF-I levels in adolescence than those without catch-up.

Disclosure: Was this work supported by industry? Yes: Novo Nordisk A/S, Lundbeck foundation.
Do you act as a consultant, employee or shareholder with this industry? No.

B-17

Thin, fat, or both? An explorative study on the prevalence of the 'thin—fat' Indian baby in the Hague (The Netherlands)

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Background: Diabetes prevalence is extremely high among South Asians all over the world, including The Netherlands. The causes of this high prevalence are unclear. One of the proposed theories is the fetal origins-hypothesis, which proposes that persistent metabolic and structural changes caused by fetal undernutrition increase the risk of type 2 diabetes and cardiovascular disease in later life. A striking finding in Indian babies is the combination of a low birth weight and a high body fat percentage (the 'thin—fat' baby). Moreover, Indian babies are characterized by hyperinsulinemia already at birth. However, this is unknown for South Asian babies in western countries, although the prevalence of diabetes among South Asians is even higher in western countries.

Objectives: To assess the prevalence of the thin-fat insulin resistant phenotype in South Asian neonates in The Hague.
Methods: Summer 2006 we will start a prevalence study in The Hague. We will measure weight, crown-heel length, abdominal, and mid-upper arm circumference and thickness of triceps and subscapular skinfold of 100 South Asian and 200 Dutch babies within 72 h after birth. Also, at delivery, cord blood samples will be taken to determine insulin, glucose, and triglycerides levels.
Future plans: This project is a pilot study for a large-scale study in which we will assess associations between the nutritional status of the mother and anthropometric and metabolic parameters of the neonate. Our study is based on the design of the Pune Maternal Nutrition Study, in which associations were found between the nutritional status of the mother and neonatal anthropometry in India.

Disclosure: Was this work supported by industry? No.

B-18

A population-based birth-cohort study within the Groningen Expert Center for Kids with Obesity (GECKO Drenthe)

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Objective: The aim of the population-based birth-cohort study within GECKO is to study the etiology and prognosis of overweight and the metabolic syndrome during childhood.
Methods: The GECKO Drenthe will be a population-based observational birth-cohort study, which includes all children born from April 2006 to April 2007 in Drenthe, one of the northern provinces of The Netherlands. During the first year of life, the study includes repeated questionnaires, extensive anthropometric measurements, and blood measurements at birth (cord blood) and at the age of 9 months.
Results: The number of babies born in the Drenthe province is about 5.500 per year. The preliminary results concerning the participants will be shown.
Conclusion: GECKO Drenthe is a unique project that will contribute to the understanding of the development of obesity in childhood and its tracking into adulthood. This will enable early identification of children at risk and opens the way for timely and tailored preventive interventions.

Disclosure: Was this work supported by industry? Yes: Hutchison Whampoa Ltd.
Do you act as a consultant, employee or shareholder with this industry? No.

B-19

Body proportions and blood pressure at age 3 years: reverse associations with upper and lower body height

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Background: In adults, shorter leg length is associated with higher blood pressure. In children, height is positively correlated with blood pressure, but components of height have not been investigated.
Objective: To compare associations between components of height and blood pressure at age 3 years.
Methods: Using an oscillometric automated recorder, we obtained up to five measures of blood pressure on a single occasion at the 3-year examination (2003—2006) of 1133 children participating in Project Viva, a US cohort study of pregnant women and their offspring. We estimated covariate-adjusted associations of leg and trunk length with blood pressure, before and after adjusting for height.
Results: Mean systolic (SBP), diastolic (DBP), and mean arterial pressures (MAP) were 92.1, 58.1, and 69.4 mm Hg in girls and 92.4, 58.3, and 69.7 mm Hg in boys. Adjusting for age and sex, leg and trunk length were each positively associated with SBP (0.32 [95% CI 0.09, 0.55] and 0.74 [0.52, 0.96], respectively, for a 1 cm increment in height component). After further adjustment for height, leg length was inversely associated with SBP (−0.61 [−0.98, −0.24]), whereas trunk length was still directly associated (0.61 [0.24, 0.98]). We found similar results for DBP and MAP. Adjustment for weight partially attenuated these estimates. Analysis of leg percent or difference in leg and trunk *z*-scores yielded similar results.
Conclusions: Poor growth of the lower body, relative to total body height, is associated with higher blood pressure at age 3 years. Factors limiting early growth of the long bones may determine an individual's blood pressure trajectory.

Disclosure: Was this work supported by industry? No.

B-20

A population-based cohort study among adolescents evaluating risk factors for obesity and the metabolic syndrome—the GECKO-TRAILS study

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Objective: The aim of this study is to assess childhood predictors of insulin resistance and the metabolic syndrome in adolescence.
Methods: The GECKO-TRAILS is a population-based cohort study among 2200 adolescents, aged 14—16 years. They have been assessed biennially from the age of 10 within the framework of TRAILS (Tracking of Adolescents' Individual Lives Survey). Amongst others weight and height from birth are being obtained. During the current assessment, additional data are being obtained in collaboration with the GECKO (Groningen Expert Center for Kids with Obesity), including questionnaires, anthropometry, and body composition and a fasting venapuncture.
Results: Preliminary results in 55 adolescents aged 15—16 years show a prevalence of overweight and obesity of 16.4% and 3.6%, respectively. Mean BMI was 21.0 kg/m^2 with a range of 15.5 to 35.2 kg/m^2. Mean fasting insulin was 15.0 mU/l with a range of 6.0 mU/l to 56.0 mU/l. These figures indicate that

a considerable variation exists within our population. Further preliminary results in around 500 children will be presented.
Conclusion: The GECKO-TRAILS study will provide insight in risk factors for insulin resistance and the metabolic syndrome in adolescence.

Disclosure: Was this work supported by industry? Yes: Hutchison Whampoa Ltd.
Do you act as a consultant, employee or shareholder with this industry? No.

B-21
Comparison of birth weight distributions from Santiago, Chile

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Objective: A recent technical report from the World Health Organization has proposed the development of birth weight references for each population based on observations of low-risk healthy pregnant women and their neonates (Promoting optimal fetal development. Geneva: WHO, 2006). We report a comparison of birth weight distributions from newborns delivered by pregnant women in Santiago, Chile. Healthy subjects were compared to the total sample.
Materials/methods: Prospective information from all women delivering in years 2000—2004 was collected at one of the biggest public Maternity Hospitals from Santiago (Sótero del Río Hospital). Women delivering single pregnancies at weeks 39—41, without pathologies or behaviors that may affect fetal growth were selected as healthy population. Descriptive statistics of the total population and the healthy population were calculated.
Results: A total of 28,897 newborns were studied. Healthy selected pregnancies resulted in 12,300 newborns. In the total sample, mean birth weight was 3314 ± 586 g, skewness -1.065, and kurtosis 3.299. In the healthy population, mean birth weight was 3505 ± 414 g, skewness 0.183, and kurtosis 0.728.
Conclusions: A higher concentration of preterm births and specific maternal pathologies in the total population is interpreted as the cause for the observed skewness to the left in the birth weight distribution. Birth weight distribution of the healthy selected pregnancies was normal and it can be considered as an adequate standard for the population of newborns studied. Therefore, this reference distribution might be an adequate target for health interventions.

Disclosure: Was this work supported by industry? No.

B-22
Completeness of Chilean information on live births: design of a diagnostic instrument relating birth weight and gestational age at birth to neonatal mortality

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Objectives: To determine the combined effect of birth weight (BW) and gestational age at birth (GA) on neonatal mortality using individually identified live births.
Materials/methods: We used logistic regression to study the interactive effect of BW and GA on the individual probability of neonatal death. All live births from Chile in year 2000 were included in a linked file in which the recent use of individual identification number permits the matching of information from the live births and the neonatal deaths: 248,893 live hospital births who survived the neonatal period and 1463 neonatal deaths were included. Odd ratio (OR) models for BW and also for GA were developed for each sex using a smoothing function. The probability of neonatal death by gender was presented using contour plots.
Results: All live births had identification number but 99.82% of all survivors and 97.4% of all neonatal deaths had complete information on BW, GA, and gender. Results of the univariate and multivariate analyses demonstrated that models were statistically significant and ORs were different and non-linear for the effects of BW and GA. Correspondingly, contour plots of constant neonatal mortality according to BW and GA were presented; they were similar for each sex. Therefore, they were presented in a single graph for both sexes.
Conclusions: A diagnostic graph was developed which can be an effective clinical tool to assess risk of death at birth. The complete use of identification numbers at birth since year 2000 can suit birth-cohort studies with good initial information.

Disclosure: Was this work supported by industry? No.

B-23
Developmental origins of health and disease: profile of a population from a tertiary public hospital in northeast Brazil

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Objective: To describe the profile of a maternal and neonatal population assisted at a tertiary public hospital in northeast Brazil, with emphasis in the morbidities associated with the developmental origins of health and disease.
Materials/methods: Transversal study with data from two different sources. The hospital database with all deliveries from 2003 to 2005 was used to characterize the studied population. To analyze maternal and neonatal morbidity, a review of charts from all children discharged from January to April/2006 was conducted. Results are described as simple frequencies.
Results: From January 2003 to December 2005, there were 7423 deliveries. The mothers were poor women, 26.5% were adolescents, and 2.0% were older than 39. The majority (77.3%) had no professional activity, 12.9% had less than four full school years, and 4.9% had over 12. They delivered 1877 babies (25.3%) weighting less than 2.5 kg and 334 (4.5%) over

3.9 kg; 24.7% were premature. Adolescent mothers delivered 26.9% of premature and 28.2% of low birth weight babies. From January to April 2006, 864 neonates left the hospital. There were 17.6% pre-term, 0.2% post-term, 6.6% SGA, and 9.9% LGA babies. The proportion between prematurity and SGA (12.5%) was higher than among the term babies (5.2%) and the opposite occurred in relation to LGA (0.7% in pre-terms and 11.9% among term babies). Hypertension was detected in 45.5% of mothers and diabetes in 2.0%.
Conclusions: Risk factors for the development of adult chronic diseases are highly frequent in our maternal—neonatal population. A DOHaD centre can help to elucidate underlying mechanisms and establish new preventive strategies.

Disclosure: Was this work supported by industry? No.

B-24
Growth of very low birth weight infants

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Objective: To evaluate risk factors for growth failure at 1 year corrected age of newborns with birth weight ≤1500 g.
Methods: A cohort of newborns with birth weight ≤1500 g admitted between 11/2003 to 12/2004 was followed up to 12 months corrected age. Weight, length, and head circumference were plotted on NCHS curves. The group was divided in relation to *Z*-score: <2 S.D. and ≥2 S.D. Birth weight, gestational age, adequacy to gestational age, length of hospital stay, maternal education, and hospital readmission were compared between both groups. Birth weight ≤750 g, presence of PIVH and/or PVL, clinical sepsis, and maternal education ≤9 years were also analyzed.
Results: 116 patients were admitted, 32 died, 14 never returned to follow-up clinic, 5 died during the follow, and 11 were lost. 54 newborns were included in the study. Head circumference, length, and weight were <2 S.D. in 6, 7, and 9 newborns, respectively. No studied risk factor was associated to low head circumference or length. Low gestational age was associated to weight <2 S.D. ($p = 0.024$). There was a significant statistical association between 1 year low weight and birth weight ≤750 g, presence of PIVH and/or PVL, clinical sepsis, and maternal education ≤9 years ($p = 0.037$).
Conclusions: Birth weight ≤750 g, presence of PIVH and/or PVL, clinical sepsis, and maternal education ≤9 years are important risk factors for growth failure in VLBW infants.

Disclosure: Was this work supported by industry? No.

B-25
Effects of maternal smoking in pregnancy on prenatal brain development. the Generation R Study

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Objective: Nicotine is considered to be a neuroteratogen, even in concentrations that do not cause intrauterine growth retardation. This study aims to examine the associations of maternal smoking in pregnancy with fetal growth of the head and specific brain development parameters.
Methods: This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until adulthood. Maternal smoking was assessed by questionnaires in early pregnancy (gestational age <18 weeks), mid-pregnancy (gestational age 18—25 weeks), and late pregnancy (gestational age ≥25 weeks). Head circumference, biparietal diameter, transcerebellar diameter, and atrial width of lateral ventricle were measured in late pregnancy. The analysis on fetal head growth was based on 7998 subjects. Transcerebellar diameter and the atrial width of lateral ventricle were measured in late pregnancy in 6434 and 4712 subjects, respectively.
Results: Fetuses of mothers who continued to smoke during pregnancy had 1.98 (95% confidence interval: −2.62, −1.35) mm smaller head circumference and 0.56 (95% confidence interval: −0.78, −0.35) mm smaller biparietal diameter compared to fetuses of mothers who never smoked during pregnancy. These effect estimates were adjusted for fetal gender, maternal educational level, maternal ethnicity, parity, maternal alcohol consumption, and maternal anthropometrics. Maternal smoking during pregnancy was not associated with differences in transcerebellar diameter or atrial width of lateral ventricle.
Conclusion: Continuing smoking during pregnancy leads to decreased growth of the head. There is no evidence from this study for a specific vulnerability of brain structures to nicotine.

Disclosure: Was this work supported by industry? No.

B-26
Familiar aggregation of hypospaidias—a cohort study

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Objectives: To investigate the familiar contribution to the risk of developing hypospadias in a population-based cohort of Danish males.
Materials/methods: Using Danish health registers, we identified 5119 infants with a diagnosis of hypospadias in a cohort of 1,201,790 boys born during 1973—2004. A multigenerational register based on the Danish Civil Registration System was used to estimate the familial aggregation of

hypospadias within first-, second-, and third-degree relatives. Binomial logistic regression analysis was used to calculate the recurrence risk ratio (RRR) for relatives of an index case.
Results: Among 5119 hypospadias cases, 197 (3.85%) had a family history of hypospadias. After adjusting for confounders, the RRR of hypospadias in same-sex twins, first-, second-, and third-degree relatives of an index case was 72.7 (51.4—103.0), 10.5 (8.7—12.7), 2.3 (1.7—3.3), and 1.3 (0.9—1.7) respectively. The RRR among first-degree relatives was higher among brothers 12.2 (9.8—15.1) than for offspring of an index case 7.7 (5.3—11.2). We found no difference in RRR in paternal vs. maternal second- and third-degree relatives.
Conclusion: The results suggest that inherited genetic factors have a strong effect on the inheritability of hypospadias resulting in an increased risk in first-, second- and third-degree relatives of an index case. Further, we found that the maternal and the paternal line contribute equally to the inheritance of hypospadias.

Disclosure: Was this work supported by industry? No.

B-27
Is leg length a biomarker of childhood conditions in Chinese women? The Guangzhou Biobank Cohort Study

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Objective: In developed western populations, longer legs have been shown to be a biomarker of better early childhood conditions. However, we hypothesised that in transitioning populations better childhood conditions may bring forward puberty and thus decrease leg length, counteracting the overall positive effect of a favourable childhood environment on leg growth.
Methods: Structural equation modelling was used to assess the interrelation of age, education, father's job, age of menarche, and leg length in a cross-sectional sample from 2003 to 2004 of 7273 Chinese women aged at least 50 years from the Guangzhou Biobank Cohort Study.
Results: Leg length had no significant association with education or father's occupation on bivariable testing. After including age of menarche in the model, education was associated with longer legs (0.045 cm/year of education, 95% confidence interval (CI) 0.02 to 0.07). Education was also associated with younger age of menarche (0.12 cm/year of education, 95% CI 0.11 to 0.13), which was in turn associated with shorter legs (0.23 cm/year of menarche earlier 95% CI 0.18 to 0.27).
Conclusion: In the rapidly developing population of Guangzhou, leg length in women may not be a reliable marker of childhood conditions, because of the opposing effects of improved childhood conditions on growth and tempo of maturation.

Disclosure: Was this work supported by industry? No.

B-28
Explaining the association between low maternal education and increased risk of gestational diabetes; the Generation R Study

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Objective: Several studies have shown an association between lower maternal education and gestational diabetes. The aim of this study was to determine to what extent this relation can be explained by lifestyle factors, including smoking, alcohol consumption, and pre-pregnancy body mass index (BMI).
Materials/methods: This study was embedded in The Generation R Study, a population-based prospective cohort study from fetal life until adulthood. Maternal education was assessed in early pregnancy (around 12 weeks gestation) and divided into five categories. The presence of gestational diabetes was assessed by questionnaire in late pregnancy (around 30 weeks). All confounders and intermediary factors were assessed by questionnaire in early pregnancy. Odds ratios of gestational diabetes were calculated using logistic regression analysis among 8880 subjects.
Results: Women with only primary education had a higher risk of gestational diabetes (OR 3.59, 95% CI: 1.28—10.02) compared to women with university education after adjustment for ethnicity, parity, age, family and obstetric history, and pre-existent diabetes and hypertension. Additional adjustment for lifestyle factors (smoking before and during pregnancy, alcohol consumption, pre-pregnancy BMI) reduced the OR to 1.42 (95% CI: 0.38—5.25). The attenuation in odds ratio was almost completely due to the introduction of BMI to the model.
Conclusion: Our results show an association between lower maternal education and gestational diabetes, which is largely explained by pre-pregnancy BMI.

Disclosure: Was this work supported by industry? No.

B-29
Low maternal education is a risk factor for pregnancy-induced hypertension; the Generation R Study

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Objective: The aim of this study was to examine whether low maternal education is associated with pregnancy-induced hypertension.
Materials/methods: This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until adulthood. Maternal education was assessed in early pregnancy (around 12 weeks gestation) and divided into five categories. The presence of pregnancy-induced hypertension was assessed by questionnaire in late

pregnancy (around 30 weeks). All confounders and intermediary factors were assessed by questionnaire in early pregnancy. Odds ratios of pregnancy-induced hypertension were calculated using logistic regression analysis among 8880 subjects.
Results: Women with only primary education had a higher risk of pregnancy-induced hypertension (OR 3.62, 95% CI: 1.70—7.69) compared to women with university education after adjustment for ethnicity, parity, age, family and obstetric history, and pre-existent hypertension and diabetes. After additional adjustment for possible intermediary factors (smoking, alcohol consumption, and pre-pregnancy body mass index (BMI), the OR was 3.10 (95% CI: 1.21—7.93). The attenuation was largely due to the effect of pre-pregnancy BMI.
Conclusions: This study shows that low education is associated with a higher risk of pregnancy-induced hypertension. Pre-pregnancy BMI explains a large part of this increased risk. Since pregnancy-induced hypertension has short- and long-term adverse effects on health of both mother and child, future research should focus to further explain educational inequalities in pregnancy-induced hypertension.

Disclosure: Was this work supported by industry? No.

B-30
Effects of working conditions on birth weight: the ABCD-study

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Objective: Growing evidence shows that adverse maternal life style factors, early in pregnancy, play a pivotal role in fetal programming with subsequent adverse effect on birth weight and beyond. This study investigates the birth weight effects of a wide array of working conditions during first trimester in a large prospective cohort (ABCD-cohort).
Methods: All pregnant Amsterdam women [7/1/03—7/3/04 (N=12.377), response 8266 (67%)] were invited to fill out a detailed questionnaire, 2 weeks after prenatal screening. Employment was defined as paid work $\geq$8 h/week during first trimester. Working conditions were: working hours/week, standing/walking hours/week, physical work load, and job-strain (Karasek Demand-Control model). Only viable singleton deliveries with pregnancy duration $\geq$37 weeks were included (N=7141).
Results: Linear regression revealed that unemployment (37% of cohort) was associated with lower birth weight (on average −45 g). Heavy working conditions showed the same: high physical work load: −85 g, standing/walking >20 h/week: −45 g, working $\geq$32 h/week: −90 g, and high job-strain −112 g (ref lowest working condition). After adjusting for confounders (pregnancy duration, gender, parity, smoking, maternal length, maternal BMI, education level, marital status), only weekly working hours (−47 g) and job strain (−68 g) remained significant. The interaction of working $\geq$32 h/week with high job-strain showed the largest reduction (−140 g), cf. the effect of smoking.
Conclusion: Job-strain early in pregnancy significantly lowers birth weight in particular if working $\geq$32 h/week.

Disclosure: Was this work supported by industry? No.

B-31
Early childhood weight gain predicts weight at 9 years in VLBW children

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Objective: To determine if specific periods of growth [birth to 1 year, 1 to 4 years, and 4 to 9 years] were associated with subsequent weight and body mass index (BMI) in prematurely born very low birth weight (VLBW) children at age 9.
Methods: Sixty-eight VLBW children who participated in a randomized controlled trial of postnatal dexamethasone were assessed at 1, 4, and 9 years of age for measurement of height and weight, and from these measurements BMI was calculated. *Z*-values were determined from national reference data. Weight gain was determined from the change in weight *z*-values (?Wtz) across time periods.
Results: No differences were observed between treatment groups for anthropometric variables at birth, 1, 4, or 9 years. Median *z*-values (range in parenthesis) at 9 years were 0.15 (−2.73, 2.79) for weight and 0.13 (−2.00, 2.37) for BMI. Thirty percent of the children were at risk for overweight or overweight at 9 years. Change in weight *z*-values were $\Delta Wtz_{1yr\text{-}b}$ − 1.01 (−3.77, 2.79), $\Delta Wtz_{4\text{-}1yr}$ 0.43 (−3.49, 5.33), and $\Delta Wtz_{9\text{-}4yr}$ 0.73 (−0.99, 2.96). The $\Delta Wtz_{4\text{-}1yr}$ was correlated with current weight (r_s=0.490) and BMI (r_s=0.563) at 9 years of age, as was the $\Delta Wtz_{9\text{-}4yr}$ (r_s=0.388) and (r_s=0.364), respectively. The $\Delta Wtz_{1yr\text{-}b}$ was not associated with anthropometrics at 9 years of age.
Conclusion: In this cohort of VLBW children weight gain from 1 to 4 years appeared to be more strongly predictive of weight and BMI at 9 years of age than weight change during infancy.

Disclosure: Was this work supported by industry? No.

B-32
Birth size, adult body composition, and muscle strength in later life

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Objective: Low birth weight has been linked to lower lean body mass and abdominal obesity later in life, while high birth weight has been suggested to predict later obesity as indicated by high body mass index (BMI). We examined how birth weight was related to adult body size, body composition, and isometric grip strength.
Methods: Lean and fat mass were estimated by a multi-frequency bioelectrical impedance analysis with eight tactile electrodes in 928 men and 1075 women born in 1934—1944, with measurements at birth recorded.

Results: A 1 kg increase in birth weight corresponded in men to a 4.1 kg (95% CI: 3.1, 5.1) and in women to a 2.9 kg (2.1, 3.6) increase in adult lean mass. This association remained significant after adjustment for age, adult height or BMI, physical activity, smoking status, and social class. Grip strength was positively related to birth weight through its association with lean mass. The positive association of birth weight with adult BMI was a reflection of differences in lean mass. Accordingly, the relationship between low birth weight and higher fat percentage at a given level of adult BMI was attributable to a lesser amount of lean mass. Abdominal obesity was not predicted by low birth weight.
Conclusions: Low birth weight contributes to the risk of relative sarcopenia and the related functional inability at the other end of the lifespan. We question the relevancy of BMI as an index of fatness in studies assessing the relationship between birth weight and obesity.

Disclosure: Was this work supported by industry? No.

B-33
The relation of birth weight and lung function during adulthood: a cohort study

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Objective: To examine the relationship between birth weight and lung function during adulthood in China.
Methods: 627 singletons were followed-up, born in the Peking Union Medical College Hospital in Beijing during 1948—1954 and blood pressure, glucose, insulin, cholesterol, forced expiratory volume (FEV1, L), forced vital capacity (FVC, L), and peak expiratory flow (PEF□L/min) were measured.
Results: (1) Prevalence of chronic obstructive pulmonary disease (COPD) was 7.7%; FEV1, FVC, PEF, and FEV1/FVC% were 2.91%, 3.64%, 447.86%, and 80.79% respectively. (2) Birth weight was divided into ≤2500, 2500—3999, ≥4000 g groups; FEV1 were 2.65, 2.93, 3.02 ($p<0.01$); FVC were 3.33, 3.66, 3.77 ($p<0.05$); PEF were 405.82, 450.08, 479.71 ($p<0.01$), respectively. While FEV1/FVC% were 80.09, 80.85, and 80.56 ($p>0.05$). Ponderal index (PI) was divided into ≤24, 24—25, 26—28, and >28 groups; COPD were 10.1%, 5.6%, 6.7%, and 10.9%, respectively. Body mass index (BMI) was divided into <24, 24—27, and ≥28 groups; COPD were 9.3%, 7.0%, and 1.5%, respectively. (3) Low birth weight (RR=2.02, 95% CI: 0.55—7.41) or lowest 25% PI (RR=1.70, 95% CI: 0.75—3.87) was not COPD risk factor by logistic regression analysis after adjustment for gestational age, lifestyle, and diseases (such as hypertension, diabetes, bronchitis, tuberculosis, pulmonary infection recently), while higher BMI (RR=0.43, 95% CI: 0.21—0.87) and female at birth was COPD protective factor (RR=0.31, 95% CI: 0.14—0.73); and asthma increased COPD risk (RR=24.25, 95%CI: 8.11—72.57).
Conclusions: Birth weight might impact on lung function during adulthood, while low birth weight or lower PI were not significantly related to COPD in this cohort study.

Disclosure: Was this work supported by industry? No.

B-34
The Generation R Study: design and cohort profile

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The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood. The study is designed to identify early environmental and genetic causes of normal and abnormal growth, development and health from fetal life until young adulthood.
The study focuses on four primary areas of research: (1) growth and physical development; (2) behavioural and cognitive development; (3) diseases in childhood; and (4) health and healthcare for pregnant women and children.
In total, 9778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study. Of all eligible children at birth, 61% participate in the study. Data collection in the prenatal phase included physical examinations, questionnaires, fetal ultrasound examinations, and biological samples. In addition, more detailed assessments are conducted in a subgroup of 1232 pregnant women and their children.
The children form a prenatally recruited birth-cohort that will be followed until young adulthood. Eventually, results forthcoming from the Generation R Study have to contribute to the development of strategies for optimizing health and healthcare for pregnant women and children.

Disclosure: Was this work supported by industry? No.

C. CARDIOVASCULAR DISEASE, HYPOXIA

C-01
Elevated BNP expression in mouse offspring left ventricles after protein restriction in utero

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Objectives: We have previously shown that cyclin G1 expression is reduced in fetal hearts after *in utero* protein restriction (PR) suggesting reduced cardiac cell cycle. However no difference in cyclin G1 expression was seen in adult offspring hearts. We hypothesised that the hearts of

adult PR group should be under greater stress to maintain cardiac output. We therefore measured brain natriuretic peptide (BNP) expression in fetal hearts and left ventricles of adult offspring in the control (C) and PR groups because BNP is a marker of left ventricular dysfunction during volume overload or cardiac fibrosis (Nishikimi et al. Cardiovasc Res. 2006).
Methods and results: Pregnant CD1 mice were placed on C (18% casein) or PR (9% casein) diet. Fetal hearts were collected on day 12 of gestation (C, $n=11$, PR, $n=10$) and the left ventricles (LV) of adult offspring at 6 months (C, $n=17$, PR, $n=17$). Fetal heart BNP mRNA expression relative to unit total RNA as measured by real-time PCR was similar in C and PR (C, 0.858 ± 0.104 vs. PR, 0.761 ± 0.096, $p=$NS). However, BNP expression in adult LV was greater in the PR than C (C, 7.043 ± 0.68 vs. PR, 11.012 ± 1.54, $p=0.04$).
Conclusion: These results indicate that protein restriction in pregnancy induces cellular changes (indicated by cyclin G1 changes) in the fetal heart which places it under stress in adulthood (elevated BNP production). Because BNP can suppress ventricular remodelling, we are presently investigating cardiac structural changes to assess whether these alterations are adaptive or maladaptive. *Supported by BUPA and BHF.*

Disclosure: Was this work supported by industry? No.

C-02
Chronic palatable hypercaloric diet in adulthood of neonatally handled rats and risk factors for later cardiovascular disease

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Objective: Brief and repeated separations from the mother in the neonatal period in rats are associated with a differential level of maternal care and altered feeding behavior in adulthood. Our objective was to verify the effects of chronic exposure to a high palatable food in neonatally handled rats, evaluating known risk factors for cardiovascular diseases such as plasma total cholesterol, HDL, triglycerides, butyrylcholinesterase (BuChE) levels, and abdominal fat deposition.
Matherials/methods: Nests of Wistar rats were (1) handled for 10 min/day in the first 10 days of life or (2) left undisturbed until weaning. At 3 months of age, females were assigned to receive or not chocolate + standard lab chow for 30 days and then sacrificed to collect serum, plasma and abdominal fat. A group of animals receiving chocolate was deprived for 30 days, receiving only rat chow before the sacrifice. Repeated measures ANOVA or two-way ANOVA were used for the analysis.
Results: Groups receiving chocolate had higher body weight, abdominal fat deposition, and triglycerides. Cholesterol and HDL did not differ between the groups. Plasma BuChE activity was increased in the intact group receiving chocolate ($p=0.028$), with no effect on the neonatally handled group. After 30 days of abstinence, the altered parameters returned to normal levels.
Conclusion: Considering plasma BuChE activity, an enzyme associated with risk for cardiovascular and Alzheimer disease, neonatal handling was protective after a chronic exposure to a high caloric diet. Further studies are necessary to better evaluate the implications of this result.

Disclosure: Was this work supported by industry? No.

C-03
Accelerated age-related decline in renal function and altered vascular function in aged female rats following gestational dietary protein restriction

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Objective: Maternal protein restriction leads to low birth weight, reduced nephron endowment, and altered vascular reactivity in young offspring, which may program for disease later in life. This study determined the effect of gestational dietary protein restriction on renal and vascular function in aged rats.
Materials/methods: Pregnant rats were fed a normal (NPD, 20% casein) or low (LPD, 8.7% casein) protein diet throughout gestation. Conscious mean arterial pressure (MAP) and anaesthetised renal function were measured via clearance methods in NPD ($n=8$, 4 male) and LPD ($n=10$, 5 male) offspring at 100 weeks of age. Vascular function and mechanical wall properties in mesenteric arteries from NPD and LPD offspring were also assessed using wire and pressure myographs.
Results: At 100 weeks of age, body weight and MAP was not different between the dietary groups. Glomerular filtration rate was 0.26 ± 0.04 ml/min/g KW (NPD-male), 0.86 ± 0.16 ml/min/g KW (NPD-female), 0.27 ± 0.04 ml/min/g KW (LPD-male), and 0.40 ± 0.13 ml/min/g KW (LPD-female), respectively ($p_{DS}=0.02$, two-way ANOVA factors diet and sex). In addition, there was reduced sensitivity to the nitric oxide donor, nitroprusside in mesenteric arteries of LPD offspring compared to NPD controls. There was a gender-dependent enhancement of wall tension generated upon pressurization of mesenteric arteries of female LPD offspring, but not in arteries from LPD male offspring when compared to NPD controls.
Conclusion: Female offspring exposed to maternal protein restriction may be at greater risk of renal failure and vascular dysfunction at old age when compared to their male counterparts.

Disclosure: Was this work supported by industry? No.

C-04
Maternal vitamin D deficiency in rats: effect on kidney and heart development in the offspring

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Objective: The aim of this study was to determine the effect of exposure to vitamin D deficiency from conception on nephron endowment and cardiomyocyte number in young rat offspring.
Materials/methods: Sprague—Dawley rat dams were fed either a vitamin D deplete or vitamin replete (control) diet for 6 weeks prior to pregnancy, during pregnancy and throughout lactation. At 3 days of age cardiomyocyte number in the hearts of their offspring is currently being analysed using an optical disector/fractionator stereological technique. In other litters the offspring have been weaned at 4 weeks of age and maintained on their respective diets until experimentation at 7 weeks of age. At necropsy, the right kidney was excised and fixed. Nephron number was determined using unbiased stereological techniques ($n=7$ per group).
Results: There was no significant difference in kidney weights between the vitamin D deplete and control offspring. Interestingly, there was a 20% increase in the number of nephrons in the kidneys of the vitamin D deplete offspring (vitamin D deficient, $29{,}003 \pm 1858$ vs. control, $23{,}334 \pm 1728$: $p=0.05$). This was accompanied by a significant decrease in glomerular size in the vitamin D deplete group compared with the controls ($6.125 \pm 0.576 \times 10^{-4}$ mm^3 and $8.178 \pm 0.247 \times 10^{-4}$ mm^3, respectively; $p=0.0066$). Stereological estimation of cardiomyocyte number in the control and vitamin D deplete rat offspring is ongoing.
Conclusion: Vitamin D deficiency *in utero* appears to stimulate nephrogenesis. However, further studies are required to elucidate the mechanisms.

Disclosure: Was this work supported by industry? No.

C-05
The effect of pre- and periconceptional undernutrition on cardiac electrophysiology in adult male sheep offspring

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Introduction: The mechanisms underlying the association between size at birth weight and cardiovascular disease in adulthood have not been determined. The induction of ventricular tachycardia (VT) and dispersion of effective refractory period (ERP) is linked to cardiac arrhythmogenesis and sudden cardiac death. We studied the effects of nutritional challenges around time of conception on cardiac electrophysiology (EP) in adult male sheep.
Methods: Welsh Mountain ewes were assigned to dietary groups prior to mating and fed 100% total nutrient requirements (control, $n=8$) or 50% total nutrient requirements 30 days prior to conception (PRE, $n=12$) or for 15 days before and after conception (PERI, $n=12$), and 100% thereafter. At 3.3 years, EP studies were conducted on the male offspring under general anaesthesia. By pacing in the right and left ventricular apex (RV and LV), we measured the dispersion of ERP (difference in ERP between RV and LV) and susceptibility to ventricular tachycardia (VT) induction, using a predefined intracardiac pacing protocol. Data (mean $\pm$ S.E.M.) were analysed by ANOVA and a Bonferroni post hoc test.
Results: VT has not induced from the RV or LV in any of the dietary groups. There were no differences in the dispersion of ERP in the control, PRE, and PERI groups (15.71 ± 3.69 vs. 10.83 ± 2.60 vs. 15.45 ± 5.62, $p=0.64$).
Conclusion: This suggests that pre- and periconceptional undernutrition in not associated with latent ventricular arrhythmogenesis or sudden cardiac death at this age. Other aetiological and developmental determinants of cardiovascular disease need to be studied.

Disclosure: Was this work supported by industry? No.

C-06
Chronic hypoxia during gestation alters mediation of hypoxia-induced vasodilatation in offspring

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Objective: Chronic hypoxia *in utero* (CHU) is associated with the developmental origins of cardiovascular diseases such as hypertension (2). CHU rats maintain a higher arterial blood pressure (ABP) during systemic hypoxia (3). In normal (N) rats, A_1 adenosine-receptors partly mediate the fall in blood pressure (4). The role of A_1-receptors in the response to systemic hypoxia in CHU rats is now investigated.
Methods: Experiments were performed on Wistar rats. A small control group ($n=4$) was used to confirm previous findings (4). For the CHU group ($n=10$), pregnant dams were housed in a hypoxic chamber (12% O_2) for days 10—20 of pregnancy and then returned to normoxia to give birth. All litters were housed under the same conditions. At ~ 8—9 weeks old, under anaesthesia, rats were instrumented for measurement of cardiovascular variables. Periods of acute systemic hypoxia were induced by changing inspired gas to a mixture containing 8% O_2 (3) before and after administration of an A_1-receptor antagonist (DPCPX).
Results: Hypoxia induced a fall in ABP and an increase in integrated FVC in both N and CHU rats. In N rats, these changes were reduced by ~ 50% following DPCPX administration; however, in CHU rats, they were unaltered. Heart rate changes were affected by DPCPX in both groups.
Conclusions: In N rats, systemic hypoxia induces hypotension and vasodilatation that is partly mediated by A_1-receptors. In CHU rats, the responses are unaffected by A_1-receptor antagonism, indicating that other mechanisms must be important in mediating their response to systemic hypoxia.

Disclosure: Was this work supported by industry? No.

C-07
Circulating glucose affects vascular reactivity in late gestation sheep fetuses

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Background: Cardiovascular control in late gestation fetuses is altered by undernutrition (Hawkins, 2000. Am. J. Physiol. 279, R340—48) and prevailing hypoglycaemia (Gardner, 2002. J. Physiol. 540, 351—66). Our aim was to relate circulating glucose to isolated vascular function in the late gestation fetal sheep, after early and late gestation undernutrition.
Methods: Welsh Mountain ewes were fed 100% total nutrient requirements (C, $n=2$), 40% for first 31 days gestation (dGA) (ER, $n=5$), or 50% from 104 dGA until post-mortem (LR, $n=4$), with 100% requirements at all other times. At 127 ± 1 dGA (term ~ 147 dGA), fetal blood glucose was measured, brachial artery dissected, and vascular reactivity assessed using noradrenaline (NA, 10 nM—100 μM), acetylcholine (ACh, 100 pM—10 μM), and sodium nitroprusside (SNP, 100 pM—10 μM). Analysis by ANOVA/Bonferroni, and linear regression.
Results: SNP-, but not ACh-, induced vasodilatation was reduced in ER compared to C ($p<0.01$) and LR ($p<0.05$) fetuses. NA-induced vasoconstriction was unaltered. For all animals, vascular sensitivity to ACh was positively related ($p<0.01$) and sensitivity to NA was inversely related ($p<0.01$) to circulating glucose. **Conclusion:** These data suggest that brachial artery vascular responses are altered in nutrient-restricted fetuses. Increased sensitivity to NA and decreased sensitivity to ACh, at lower circulating glucose levels, is likely to be associated with greater overall vasoconstriction, suggesting a link between fetal nutritional status and vascular reactivity. *Supported by BBSRC and Gerald Kerkut Trust*.

Disclosure: Was this work supported by industry? No.

C-08
Deterioration of cardiac remodeling in adult mouse offspring with undernutrition in utero

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Objectives: Evidence has emerged that fetal undernutrition is a risk factor for cardiovascular disorders in adulthood, together with genetic and environmental factors. Recently, the local expression of angiotensinogen and related bioactive substances has been demonstrated to play pivotal roles in cardiac remodeling, i.e. fibrosis and hypertrophy. The aim of the present study was to elucidate the possible involvement of the local cardiac angiotensin system in fetal undernutrition-induced cardiovascular disorders.
Methods and results: We recently developed a mouse model of undernutrition in utero by maternal food restriction, in which offspring (UN offspring: Cell Metab 1:371—8, 2005). The UN offspring showed an increase in systolic blood pressure (8 weeks of age, $p<0.05$ and 16 weeks, $p<0.01$), perivascular fibrosis of the coronary artery, cardiomegaly, and myocyte hypertrophy (16 weeks, $p<0.05$ for all), concomitant with a significant augmentation of angiotensinogen (Ang, $p<0.05$), and endothelin-1 (ET-1, $p<0.01$) mRNA expression in the left ventricles (16 weeks). In 18.5 postcoitum days, undernutrition in utero significantly elevated Ang, angiotensin-converting enzyme (ACE), and ET-1 mRNA levels in whole fetal heart tissues ($p<0.05$ for all).
Conclusions: These findings suggest that fetal undernutrition activated the local cardiac angiotensin system, which contributed, at least partly, to the development of cardiac remodeling in later life, in concert with the effects of increase in blood pressure.

Disclosure: Was this work supported by industry? No.

C-09
Long-term effects on blood pressure and renal function in fawn-hooded hypertensive rats (FHH) after perinatal treatment supporting nitric oxide (NO) availability

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Increasing perinatal NO availability, persistently reduced blood pressure (BP) in SHR (Racasan AJP 2005). Whether this works in a different model is unknown. Hence brief perinatal arginine plus antioxidants (ATCE) or the NO donor molsidomine (MOLS) were studied in FHH rats; a model of hypertension, impaired preglomerular resistance, and severe renal injury.
FHH dams received either ATCE or MOLS during pregnancy and lactation. Male and female offspring were studied (6 litters/group). Under anesthesia renal vascular resistance (RVR) was measured before and during either NOS inhibition with L-NNA or a superoxide dismutase mimetic (tempol).
Both perinatal ATCE and MOLS increased urinary excretion of NO metabolites at 8 weeks, i.e. 4 weeks after stopping treatment (all $p<0.05$), but this did not persist. Systolic BP was persistently reduced after MOLS (36-week females: 109 ± 4 vs. 137 ± 3 and males: 143 ± 4 vs. 151 ± 3 mm Hg, all $p<0.05$) and after ATCE in males (139 ± 2 mm Hg). Proteinuria was only reduced in ATCE females. Both perinatal treatments decreased GFR and renal blood flow and increased RVR at 36—42 weeks, suggesting persistent restoration of preglomerular resistance. Glomerular counts were not affected, but ATCE and MOLS caused significant decreases in glomerulosclerosis in males and females (all $p<0.05$). Systolic BP and RBF correlated positively with glomerulosclerosis (both $p<0.01$). MOLS increased sensitivity of RVR to both L-NNA and tempol.
Perinatal maneuvres in FHH can separately affect BP and proteinuria. Increasing perinatal NO availability in FHH can persistently suppress hypertension and glomerular injury and restore preglomerular resistance and intrarenal vasoactivity.

Disclosure: Was this work supported by industry? No.

C-10
Impaired coronary EDHF production in programmed newborn sheep

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Background: Early gestational exposure to glucocorticoids programs vascular abnormalities that lead to atherosclerosis in adulthood. Endothelial cells control coronary contractility via multiple factors including nitric oxide and endothelial-derived hyperpolarizing factors (EDHFs), which include H_2O_2. EDHFs activate smooth muscle potassium channels.
Hypothesis: Altered H_2O_2 production contributes to enhanced vasoconstrictor responsiveness of coronary arteries from steroid-exposed lambs.
Methods: Dexamethasone (DEX, 0.28 mg/kg/day i.v. for 48 h) was administered to pregnant ewes at 27—28 days gestation (term being 145 days). Offspring (all twins, $n=6$—8) were studied at 7—10 days of age. The responsiveness of left anterior descending (LAD) coronary arteries was evaluated using wire myography.
Results: LADs from DEX-exposed lambs displayed enhanced contractile responsiveness to both acetylcholine (Ach) and endothelin 1 (ET-1). This difference was no longer observed after endothelium removal or incubation in: (1) PEG-superoxide dismutase (SOD, 58 units/ml) + PEG-catalase (250 units/ml), (2) apamin (0.1 μM) + charybdotoxin (0.01 μM), or (3) L-NNA (0.1 mM). In each case, the response of the control vessel was increased to a level comparable to that of the DEX arteries.
Conclusion: The endothelium suppresses contractile responses to Ach and ET-1 in coronary arteries from newborn lambs. Both nitric oxide and EDHF-mediated modulation of these contractions are impaired in vessels from programmed lambs. The effect of EDHF is dependent upon production of reactive oxygen and potassium channel activation. The effect of SOD + catalase on control vessels suggests that the critical EDHF may be H_2O_2.

Disclosure: Was this work supported by industry? No.

C-11
Blood pressure in NOS3 knockout mice during pregnancy and in their offspring: effect of the intrauterine environment

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Objective: Using a transgenic mouse model we demonstrate that the intrauterine environment contributes to fetal vascular programming in later life. Our aim was to measure blood pressure in pregnant NOS3 knockout mice and in their offspring.
Materials/methods: NOS3 knockout (−/−KO) and wild type mice (WT) were bred to obtain heterozygous offspring born to KO mothers (+/−Mat) vs. WT mothers (+/−Pat). Blood pressure (BP) catheters were inserted into the aortic arch in 10—12-week-old female/male offspring and in −/−KO and WT mothers at day 10 of gestation. BP was recorded for 4 days in the offspring and until gestational day 18 in the mothers. Mean BP was calculated, Student's *t*-test, and one way ANOVA used for statistical analysis.
Results: Mean BP (mm Hg) was significantly higher from day 12 of gestation until day 18. At day 18 was: −/−KO, 127.4 ± 1.1 vs. WT, 95.1 ± 3.1. The average pup and placental weights were significantly lower in −/−KO vs. WT mothers. The mean BP (mm Hg) was significantly higher from the start of recording in −/−KO and +/−Mat offspring vs. WT and +/−Pat offspring (−/−KO, 150.9 ± 10.4; +/−Mat, $133.6.9 \pm 14.5$ vs. WT, 113.6 ± 4; +/−Pat, $118.2. \pm 7.8$), and remain higher until the end of recording.
Conclusion: NOS3 deficiency in pregnancy leads to abnormal fetal growth and hypertension in the offspring later in life. This hypertension is most likely the result of the altered fetal vascular programming that we have previously demonstrated. These findings highlight the role of the intrauterine environment in the developmental origin of adult disease.

Disclosure: Was this work supported by industry? No.

C-12
Course of idiopathic nephrotic syndrome in children born small for gestational age

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Objective: Clinical and animal studies have shown higher risk of an aggravated course of inflammatory renal diseases in childhood after intrauterine growth restriction. We therefore conducted a retrospective analysis of all cases treated with nephrotic syndrome between 1994 and 2004 in an university centre for paediatric nephrology.
Methods: A total number of 105 cases were identified, 67 children were suitable for analysis and presented with the diagnosis of idiopathic nephrotic syndrome. Seven children were born SGA, 58 were born AGA.
Results: The median age of manifestation was significantly higher in SGA than AGA children (6.8 (1.9—17.0) vs. 3.7 (1.2—17.2) years ($p<0.05$)). In all SGA children, renal biopsy was performed, only 55 % of the AGA children underwent renal biopsy. The proportion of children with minimal change glomerulopathy and focal segmental glomerulonephritis was not different between the groups. Although the number of relapses per patient and year was not different, all SGA children needed antihypertensive treatment in the course of the disease. In AGA children, only 41% received antihypertensive medication ($p<0.01$). Immunosuppressive therapy, the rate of steroid resistance and dependence, and the loss of renal function were not different.
Conclusions: Apart from the need of antihypertensive medication in all SGA children, we could not find evidence for an aggravated course of idiopathic nephrotic syndrome in former SGA children in our cohort. Further analysis is needed to proof a possible higher susceptibility for arterial hypertension in former SGA children with nephrotic syndrome.

Disclosure: Was this work supported by industry? No.

C-13
Contribution of the renin—angiotensin—aldosterone system (RAAS) activation to high salt sensitivity in stroke-prone spontaneously hypertensive rats (SHRSP)

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Objective: The renin—angiotensin—aldosterone system (RAAS) is associated with the development of blood pressure (BP) as well as salt-induced cerebral damage. As a new attempt to prevent stroke, we investigated the effect of angiotensin converting enzyme inhibitors (ACEI) on blood pressure and salt sensitivity in prepubescent stroke-prone spontaneously hypertensive rats (SHRSP) that were temporally treated with these inhibitors.
Methods: Male SHRSP, aged 6 weeks, were randomly assigned to receive drinking water or ACEI solution (captopril) or sardine peptide solution from 6 to 10 weeks of age. Sardine peptide is derived from sardine muscle hydrolysate and possesses an ACE inhibitory action. After cessation of treatment at 10 weeks of age, all rats were fed drinking water from 10 to 15 weeks. Following this, the rats were fed 1% saline until the end of experiment.
Results: After cessation of treatment at 15 weeks of age, their BP remained below 17 mm Hg when compared with the untreated group. The BP was elevated by salt loading in the untreated and treated groups. Compared with the untreated group, the captopril and sardine peptide groups showed reduced tissue (kidney, aorta, and mesentery) ACE activity. The life span of rats in the captopril and sardine peptide groups was greater than those in the untreated group.
Conclusion: The reduction in RAAS activation during the development of hypertension in young SHRSP may play an important role in stroke prevention.

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Do you act as a consultant, employee or shareholder with this industry? No.

C-14
Maternal protein restriction induced changes in the renin—angiotensin—aldosterone system (RAAS) during the suckling period and caused salt sensitivity in stroke-prone spontaneously hypertensive rats (SHRSP)

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Objective: The suckling period is an important stage in determining salt sensitivity in stroke-prone spontaneously hypertensive rats (SHRSP). In this study, we report that maternal protein restriction in SHRSP induces salt-sensitive hypertension and results in a shortened life span. This study investigated the effect of maternal protein restriction on the RAAS in low-protein exposed SHRSP during the suckling period.
Methods: From the day of conception, female rats were fed a 20% casein diet (CON) or a 9% casein diet (LP) until the end of pregnancy. At 15 and 28 days after birth, renal AT_1 and AT_2 receptor protein expression and plasma aldosterone concentrations were assayed.
Results: A dynamic change in the RAAS was detected at 15 days after birth. The renal AT_1 and AT_2 protein expression was significantly decreased at 28 days after birth compared with that at 15 days after birth; however, the expression levels did not differ between CON and LP. Plasma aldosterone levels at 28 days after birth were elevated as compared to those at 15 days after birth. Plasma aldosterone levels of rats in the LP group were lower than those in the CON group at 15 days after birth; however, these levels were higher than those in the CON group at 28 days after birth.
Conclusion: Maternal protein restriction induced the derangement of the RAAS during the suckling period and caused salt sensitivity in SHRSP.

Disclosure: Was this work supported by industry? No.

C-15
The relationship between gestational age and pulse pressure in children

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Objective: This study addressed the hypothesis that reduced gestational age is associated with biomarkers of adverse cardiovascular health in childhood. A cross-sectional study was undertaken to investigate the relationship between gestational age and pulse pressure in childhood, after adjusting for mean blood pressure, blood pressure variability, and other confounding factors.
Methods: Blood pressure was measured in 937 school children, free from cardiovascular disease, aged between 6 and 16 years. Of these, 483 had gestational age recorded at birth by a paediatrician (mean 39.4 weeks, range 31—43 weeks). Pulse pressure was estimated as the difference between the 24 h mean systolic and diastolic blood pressure values.
Results: Linear regression showed a significant negative association between gestational age and log-transformed pulse pressure. Gender-specific analysis showed this relationship to be confined to girls ($n=264$) (regression coefficient log mm Hg per week of gestation -0.017, 95% CI -0.03 to -0.004, $p=0.011$). This association was independent of standardised birth weight. No association was observed between gestational age and mean systolic or diastolic blood pressure or blood pressure variability. Previous studies on this cohort reported significant associations between birth weight and systolic and diastolic blood pressure and pulse pressure all specific to girls.
Conclusion: Several studies have suggested that early life exposures influence the risk of cardiovascular disease in later life. If pulse pressure is used as a biomarker of cardiovascular health, then these data indicate that being born early can increase the risk of cardiovascular disease and that this association is independent of birth weight.

Disclosure: Was this work supported by industry? No.

C-16
Naturally occurring perinatal growth restriction programs blood pressure in adult mice

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Objective: Perinatal growth restriction is associated with the development of obesity, type 2 diabetes, and hypertension. In rats, naturally occurring growth restriction is associated with hypertension despite normal renal function. Identification of a fetal programming model within an inbred mouse strain would facilitate mechanistic understanding. We hypothesized perinatal growth-restricted mice, compared to isogenic controls, have altered body composition, renal insufficiency, impaired glucose tolerance, and hypertension.
Methods: Perinatal growth-restricted (PGR) C57Bl6 pups were identified at weaning (postnatal d21) and paired with same sex normally grown littermates ($n=8$ per group). Extracellular fluid volume (ECFV) and glomerular filtration rate (GFR) were calculated at 14 weeks. Glucose and insulin tolerance tests (GTT and ITT) were performed at 16 weeks, and tail cuff systolic blood pressures (SBP) were measured at 20 weeks.
Results: PGR pups weighed less than controls from 3 weeks (7.0 g $\pm$ 0.4 vs. 10.9 g $\pm$ 0.6, $p<0.001$) through 20 weeks (19.8 g $\pm$ 0.6 vs. 23.3 g $\pm$ 1.1, $p<0.01$) despite increased caloric intake ($p<0.05$). GFR was decreased by 30% in PGR mice ($p=0.06$) with a 25% reduction in ECFV ($p<0.01$). Although growth restriction was not associated with significant alterations during GTT or ITT, SBP was higher in PGR mice (130 mm Hg $\pm$ 5 vs. 121 mm Hg $\pm$ 4, $p<0.05$) and correlated with weight at weaning ($R=0.5$, $p<0.05$).
Conclusion: Naturally occurring perinatal growth restriction, in the absence of catch-up growth or impaired glucose tolerance, is associated with (1) altered body composition, (2) reduced GFR, and (3) increased SBP.

Disclosure: Was this work supported by industry? No.

C-17
Aldosterone mediated hypernatremia in prenatally dehydrated offspring

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Objective: To determine if maternal dehydration and malnutrition in pregnancy have effects on sodium regulation in offspring.
Materials/methods: Pregnant rats were randomized to three study groups: a control group (CTL, $n=7$) received ad libitum food and water, a dehydrated group (DEHY, $n=7$) water-restricted to produce an increment of ~6 mEq/l in plasma sodium levels, and a pair-fed, nutrient-restricted group (PF, $n=7$) food intake limited to that consumed by the dehydrated group while allowing ad libitum water intake. Following delivery, the dams were placed on ad libitum diet throughout lactation. At birth, each litter size was culled to four males and four females. Each mother continued to nurse her own pups. One male and female rat of each litter were sacrificed at 21 days of age and their blood analyzed for hematocrit and plasma sodium, osmolality, and aldosterone. Values are means $\pm$ S.E.
Results: At 21 days, the plasma sodium levels were significantly higher in DEHY pups but lower in PF group (DEHY 141.2 $\pm$ 0.4, PF 134.6 $\pm$ 0.6, CTL 138.2 $\pm$ 0.3 mEq/l, $p<0.01$) as compared to controls. Furthermore, the PF group had lower plasma osmolality than DEHY and control pups ($p<0.01$). Aldosterone levels were greater in the females from DEHY group ($p<0.01$) as compared to PF and controls.
Conclusions: Maternal dehydration results in offspring hypernatremia and elevated plasma aldosterone levels. Conversely, maternal nutrient reduction results only in hyponatremia. Thus, aldosterone may play a key role in programming sodium homeostasis in prenatally dehydrated offspring.

Disclosure: Was this work supported by industry? No.

C-18
Angiotensin II mediated programmed hypertension in maternally dehydrated and nutrient-restricted offspring

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Objective: To determine if alterations in central angiotensin II (AngII) contributes to programmed hypertension and/or osmoregulation.
Materials/methods: Pregnant rats were randomized to three study groups: control group (CTL, $n=7$) received ad libitum food and water, dehydrated group (DEHY, $n=7$) water-restricted to produce an increment of ~6 mEq/l in plasma sodium levels, and pair-fed, nutrient-restricted group (PF, $n=7$) food intake limited to that consumed by the dehydrated group while allowing ad libitum water intake. At 1 day after birth, offspring brain was weighed and AngII mRNA levels were determined. Data is presented as threshold cycle (CT) normalized to β-actin (i.e., higher amounts of signal have correspondingly lower CT values). Values are means $\pm$ S.E.
Results: At day 1, DEHY and PF pups had lower body weights ($p<0.01$) and wet brain weight ($p<0.001$) than controls. When expressed relative to the body weight, DEHY and PF pups showed a relative increase in brain weight ($p<0.001$) compared to controls. Both DEHY and PF offspring showed a four-fold decrease in AngII mRNA expression than controls ($p<0.01$).
Conclusions: Both prenatally dehydrated and prenatally nutrient-restricted offspring exhibit downregulation of AngII mRNA expression, potentially suggesting an upregulation of angiotensin receptors. The similar DEHY and PF ATII results suggest that central AngII contributes to the programmed hypertension observed in both dehydrated and nutrient-restricted offspring, though likely does not contribute to programmed plasma hypertonicity observed only in DEHY offspring.

Disclosure: Was this work supported by industry? No.

C-19
Maternal dehydration programs cardiovascular and adrenal changes in offspring

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Objective: We investigated the effects of maternal dehydration and dehydration associated anorexia (pair-fed) during pregnancy on offspring body composition.
Material/Method: Pregnant rats were randomized to three study groups: control group (CTL, $n=7$) received ad libitum food and water, dehydrated group (DEHY, $n=7$) water-restricted to produce an increment of ~6 mEq/l in plasma sodium levels, and pair-fed, nutrient-restricted group (PF, $n=7$) food intake limited to that consumed by the dehydrated group while allowing ad libitum water. Following delivery, the dams were placed on ad libitum diet. Each mother continued nursing her own pups. Body and organ weights (% of body weight) of the offspring were measured at birth and at 21 days of age.
Results: Pups from DEHY and PF groups had lower body weights at birth ($p<0.01$) but by 21 days of age showed complete catch-up growth compared to controls. At birth, DEHY and PF offspring had smaller relative pancreas and larger brain weights, though normal adrenal weights. At 21 days of age, DEHY offspring demonstrated increased relative heart ($p<0.01$) and adrenal ($p<0.05$) weights, while PF exhibited normalized organ weights compared to controls.
Conclusions: Plasma hypertonicity state during the second half of pregnancy appears to specifically affect the offspring by increasing relative weights of heart and adrenal. The absence of organ weight changes in PF offspring suggests a direct effect of plasma hypertonicity rather than nutrient restriction. These results suggest programmed alterations in offspring cardiovascular and adrenal systems in response to in utero dehydration.

Disclosure: Was this work supported by industry? No.

C-20
Does childhood environment contribute to sex differences in cardiovascular risk? Hong Kong as a natural experiment

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Objectives: Male excess risk of ischaemic heart disease (IHD) has largely emerged with economic development. We took advantage of the unique history of epidemiological transition experienced by the Chinese residents of Hong Kong to examine whether sex differences in cardiovascular risk factors related to childhood exposures.
Methods: We used linear regression in a population-based cross-sectional study from 1995/1996 of 2537 long-term Hong Kong Chinese residents to assess whether childhood environment (economically developed Hong Kong or contemporaneously undeveloped Guangdong in China) had the same relationship with IHD risk factors in men and women.
Results: For men, growing up in Hong Kong rather than Guangdong was associated with higher waist hip ratio (mean difference 0.01, 95% CI 0.001 to 0.02), higher systolic blood pressure (4.65 mm Hg, 95% CI 2.55 to 7.04), and diastolic blood pressure (2.64 mm Hg, 95% CI 1.11 to 4.17), adjusted for age, socioeconomic status, marital status, lifestyle, and medication use. In contrast, for women growing up in Hong Kong rather than Guangdong was associated with lower waist hip ratio (0.01, 95% CI 0.001 to 0.02), and was unrelated to blood pressure. These different associations by sex were significant (p-values < 0.002) and consistent across the age range.
Conclusions: We speculate that an economically developed pre-adult environment enables greater sexual dimorphism and may contribute to the higher risk of IHD in men.

Disclosure: Was this work supported by industry? No.

C-21
Respiratory outcome of preterm SGA and IUGR fetuses: the impact of maternal hypertension

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Objectives: To investigate respiratory outcome in premature small for gestational age (SGA) fetuses with or without signs of intra-uterine growth restriction (IUGR) due to placental insufficiency and with or without maternal hypertensive disease.
Methods: Retrospective study in 187 neonates with birth weight <10th percentile and gestational age <34 weeks. The IUGR subgroup was identified by umbilical artery Doppler examination.
Results: No significant difference existed in respiratory outcome between fetuses with normal (SGA) or abnormal (IUGR) Doppler examination. Within the IUGR group, the RDS-incidence (OR=5.6, 95% CI=1.7—18.2, $p=0.004$), RDS-grade (OR=6.7, 95% CI=1.2—38.5, $p=0.03$), and the need for surfactant treatment (OR=5.2, 95% CI=1.1—24.4, $p=0.04$) were significantly higher in the infants of women with HELLP syndrome as compared to those of mothers without hypertensive disease.
Conclusions: Lung maturation is not accelerated in IUGR as compared to SGA fetuses. IUGR fetuses of mothers with HELLP syndrome have a significantly poorer respiratory outcome than IUGR fetuses of healthy mothers. It is possible that fetuses of mothers with HELLP syndrome are subjected to 'oxidative stress' causing lung damage rather than lung maturation.

Disclosure: Was this work supported by industry? No.

C-22
Endothelial dysfunction in aged rats following nutrient restriction in utero

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Objectives: Previously, we have shown that our model of maternal undernutrition gives rise to offspring with a

phenotype similar to that of the metabolic syndrome (Vickers et al., 2004. Am. J. Physiol. 279, E83—87). We tested whether endothelial dysfunction, another feature of the metabolic syndrome, was also present.
Materials/methods: Wistar rats were fed either a control (AD, *ad libitum* diet) or an undernutrition diet (UN, 30% of *ad libitum*) throughout gestation until term. At 1 year of age, mesenteric arteries were mounted on a wire myograph and responses to phenylephrine, endothelin, acetylcholine (ACh), and sodium nitroprusside (SNP) assessed. Data are expressed as mean ± S.E.M. and analysis performed by *t*-test. Significance accepted if $p<0.05$.
Results: Constriction to PE was similar between the groups, whilst constriction to endothelin was significantly enhanced in the UN group (pEC_{50}: AD, 9.30 ± 0.07, $n=7$; UN, 10.01 ± 0.11, $n=8$; $p<0.001$). Maximal endothelial-dependent dilatation to ACh was significantly blunted in the UN group (% max response: AD, 72.4 ± 3.3, $n=7$; UN, 44.4 ± 4.4, $n=8$; $p<0.01$). Endothelium-independent vasodilatation to SNP did not differ between the two groups.
Conclusions: These data shows that undernutrition during pregnancy leads to endothelial dysfunction at 1 year of age, apparently without changes in the vascular smooth muscle. This confirms our model has striking similarities to the human condition of the metabolic syndrome.
Supported by the NRCGD and the BHF.

Disclosure: Was this work supported by industry? Yes.

C-23
Pre- and periconceptional nutrient restriction affect coronary vascular reactivity in sheep offspring

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Undernutrition in early gestation alters cardiovascular control and coronary artery vasoreactivity in adulthood (Cleal et al., 2004. J. Physiol. 565P: C156; Khan et al., 2005. Ped. Res. 58, 1043—1044). Moreover, pre-pregnancy maternal nutrition affects fetal blood flow distribution (Haugen et al., 2005. Circ Res. 96: 12—14), but little is known of pre- or periconceptional undernutrition effects on long-term cardiovascular function.
Welsh Mountain ewes were fed 100% nutrient requirements (C, $n=8$), 50% nutrient requirements for 30 days prior to conception (PRE, $n=12$), or 50% total nutrient requirement for 15 days either side of conception (PERI, $n=12$) and 100% thereafter. 3.5-year-old female offspring were sacrificed and coronary artery reactivity to acetylcholine (ACh), thromboxane (U46619), bradykinin, isoprenaline, and sodium nitroprusside (SNP) assessed by wire myography. Data are mean ± S.E.M. and analysed by ANOVA and Bonferroni post hoc tests.
Vasoconstriction was enhanced in PRE and PERI groups to ACh (C, 86.5 ± 4.1%, $n=7$ vs. PRE, 143.1 ± 5. 1%, $n=10$ and PERI, 153.7 ± 12.0%, $n=12$; $p<0.01$), in the PERI group to U46619 (C, 63.8 ± 11.9%, $n=7$ and PRE, 43.2 ± 6.6%, $n=10$ vs. PERI, 104.2 ± 7.3%, $n=12$; $p<0.01$). Vasodilatation to bradykinin and SNP was similar between groups. Vasodilatation to isoprenaline was blunted in PRE and PERI groups (C, 95.5 ± 2.6%, $n=7$ vs. PRE, 83.7 ± 2.8%, $n=9$ and PERI, 82.7 ± 2.6%, $n=11$; $p<0.05$).
Moderate changes in maternal nutrient environment during very early development, even pre-pregnancy, can lead to enhanced constrictions and impaired vasodilatation of the adult coronary artery.
Supported by the BHF and HOPE.

Disclosure: Was this work supported by industry? No.

D. (DUTCH) FAMINE STUDIES

D-01
Reproductive outcomes after prenatal exposure to the Dutch famine of 1944—1945

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Objectives: To use the circumstances of the Dutch famine of 1944—1945 to assess reproductive outcomes in men and women in relation to prenatal nutrition deprivation.
Materials and methods: We located (1) men and women born in three institutions in the western Netherlands between January 1945 and March 1946 with prenatal famine exposure; (2) men and women born in the same institutions during 1943 or 1947 with no prenatal famine exposure as time controls (101 women, 80 men), and unexposed same-sex siblings of subjects in the above groups as sibling controls (188 women, 136 men). We defined windows of gestational exposure (gestational weeks 1—10 (50 women, 41 men), 11—20 (81 women, 67 men), 21—30 (81 women, 79 men), and 31 through delivery (82 women, 64 men) based on exposure to a ration <900 kcal/day during the whole 10-week interval. Data on offspring were collected from the study subjects during telephone interviews conducted between 2003 and 2005. Outcomes examined include number of offspring, parent's age at birth of offspring, interpregnancy intervals, birth weights for all liveborn singleton offspring, and offspring gender.
Results: There was no association between famine exposure period in gestation and any of the above outcomes, regardless of the choice of controls, although estimates lacked precision. The variability in offspring birth weights across parental exposure groups showed no clear pattern. Data reported by men and women were consistent.
Conclusion: Prenatal nutrition appears not to be associated with differences in adult reproductive performance. More precise estimates will require larger studies.

Disclosure: Was this work supported by industry? No.

D-02
Mental health at age 59 after prenatal exposure to the Dutch famine of 1944—1945

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Objective: We previously reported that adults who were exposed to the Dutch Famine during gestation had lower levels of perceived mental health as measured by the Short Form 36 Questionnaire (SF-36) Mental Component Score (MCS). We now further investigate specific domains of mental health to see if they are equally affected by prenatal famine exposure.
Methods: Six domains of mental health: level of anger as measured by the Spielberger State-Trait Anger scale, sleep quality as measured by NHANES I sleep module, and the four subscales of the SF-36 MCS (vitality, social functioning, role-emotion, and mental health) were compared between controls and those exposed to under-nutrition during gestation. Prenatal under-nutrition was based on an estimated maternal intake of $<$900 kcal/day during the famine. Four (partially overlapping) exposure groups were defined by gestational weeks exposed: 1—10 (n=85), 11—20 (n=140), 21—30 (n=157), and 31 through delivery (n=142). Unexposed controls (n=176) were born in either 1943 or in 1947 and had no prenatal exposure to the famine.
Results: Compared to the unexposed controls, exposed groups generally had worse mental health. A p-value of $<$0.05 was found for the following group differences: For the group exposed during 1—10 weeks gestation, scores were higher for level of state anger, and amount of energy (vitality) was lower. Groups exposed 1—10 weeks and 11—20 weeks were more likely to report emotions interfering with work or regular activities (role-emotion).
Conclusion: Not all domains of mental health are affected equally by prenatal famine exposure.

Disclosure: Was this work supported by industry? No.

D-03
Carotid arterial stiffness in adults after prenatal exposure to the Dutch famine

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Background: Prenatal undernutrition is associated with increases in cardiovascular disease and its biological risk factors. We investigated whether prenatal undernutrition leads to increased arterial stiffness.
Methods: We measured carotid artery lumen diameter (LD), distensibility (DC) stiffness (β), and compliance (CC) by M-mode ultrasound in 673 people, aged 56—61, who had been born as term singletons around the time of the 1944—1945 Dutch famine. Four-hundred and twelve people were unexposed and 261 were exposed to famine in utero.
Results: Exposure to famine during late (DC $24.8 \cdot 10^{-3}$/kPa; β 6.1; CC 0.74 mm^2/kPa), mid- (DC $27.0 \cdot 10^{-3}$/kPa; β 5.9; CC 0.74 mm^2/kPa), or early gestation (DC $26.0 \cdot 10^{-3}$/kPa; β 5.8; CC 0.69 mm^2/kPa) was not associated with increased carotid arterial stiffness compared to people who were unexposed (DC $25.5 \cdot 10^{-3}$/kPa, S.D. 8.6; β 6.0, S.D. 1.9; CC 0.77 mm^2/kPa S.D.; all p adjusted for gender $>$0.3). Low birth weight was associated with decreased CC ($-$0.03 mm^2/kPa for $<$2.75 kg, p adjusted for sex=0.03), but not with increased β or decreased DC. The association of birth weight with CC diminished after adjusting for LD.
Conclusion: Prenatal exposure to famine is not associated with increased carotid arterial stiffness. Low birth weight is associated with decreased carotid compliance, probably because of its association with smaller LD. Our findings do not support the hypothesis that prenatal undernutrition or low birth weight lead to decreased carotid arterial wall elastin endowment at birth.
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Disclosure: Was this work supported by industry? No.

D-04
Female fertility after prenatal exposure to the Dutch famine

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Objectives: Poor prenatal nutrition may affect fertility and reproductive success. We investigated whether prenatal exposure to the Dutch famine during different periods of gestation affects female fertility and reproductive success.
Methods: The study was carried out in a cohort of 1174 women born as term singletons around the time of the 1944—1945 Dutch famine. 475 women participated at age 56 to 61. We recorded age at menarche, at spontaneous menopause, at delivery of first child, number of children and proportion of childlessness. We compared these measures of fertility among women exposed to famine in late (n=82), mid- (n=77), or early (n=46) gestation to unexposed women (n=270).
Results: Women exposed to famine were less likely to be childless (odds ratio 0.6 [95% CI 0.3 to 1.0]) and they had more children (mean n=2.0 children) compared to women unexposed to famine *in utero* (mean n=1.7 children, p=0.02, Mann—Whitney). Women exposed to famine in mid- (hazard ratio 1.3 [95% CI 1.0 to 1.6]) or early gestation (hazard ratio 1.5 [95% CI 1.1 to 2.1]) were younger at the time of delivery of their first child compared to unexposed women. Women who were exposed in mid-gestation were 2.2 years older at menopause than unexposed women (hazard ratio for spontaneous menopause 0.72 [95% CI 0.52 to 0.98]).

Conclusions: Our findings indicate that exposure to famine in utero may affect fertility. Evidence from animal experiments suggests the underlying mechanisms may include programming of the hypothalamic pituitary gonadal axis and ovarian programming.

Disclosure: Was this work supported by industry? No.

D-05

Reduced intima media thickness in adults after prenatal exposure to the Dutch famine

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Background: Restricted prenatal growth is associated with an increased risk of coronary heart disease morbidity and mortality. We studied the effects of exposure to famine during gestation on intima media thickness (IMT) in later life.
Methods: We studied 730 people aged 58 years who were born as term singletons around the time of the 1944—1945 Dutch famine.
Results: Persons exposed to famine during gestation ($n=293$) had reduced carotid artery IMT compared to people who had not been exposed to famine in utero ($n=437$) (mean 0.71 mm, S.D. 0.16 mm compared to 0.75 mm, S.D. 0.15 mm, sex adjusted $p=0.001$). Femoral artery IMT was also thinner among people exposed to famine during gestation compared to people unexposed in utero (mean 0.64 mm, S.D. 0.20 mm, compared to 0.68 mm, S.D. 0.24, sex adjusted $p=0.07$), although the difference did not achieve statistical significance. People exposed to famine in utero developed coronary heart disease at lower carotid IMTs than unexposed people ($p=0.07$).
Conclusion: Exposure to famine in utero may reduce IMT. However, it does not reduce the risk of coronary heart disease among famine exposed people.
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Disclosure: Was this work supported by industry? No.

D-06

Stress response in adults prenatally exposed to the Dutch famine

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Stress response in adults prenatally exposed to the Dutch famine.
Objective: There is increasing evidence that restricted prenatal growth is associated with an exaggerated response to stress in later life. People who were small at birth show increased blood pressure and cortisol responses during stress. Prenatal exposure to the Dutch famine is associated with impaired glucose tolerance and an increased prevalence of type 2 diabetes and coronary heart disease. We investigated whether prenatal exposure to famine leads to an increased stress response.
Materials/methods: We measured blood pressure and salivary cortisol response to a psychological stress protocol in 725 men and women, aged 58, born as term singletons in Amsterdam around the Dutch 1944—1945 famine. Additionally, we assessed plasma cortisol concentrations after a dexamethasone suppression and an $ACTH_{1-24}$ stimulation test in a group of 98 randomly sampled cohort members.
Results: Participants exposed to famine in early gestation showed increased blood pressure response during the stress protocol compared to those unexposed to famine during gestation (4 mm Hg extra systolic increase, $p=0.04$; 1 mm Hg diastolic increase, $p=0.01$, both adjusted for gender). Salivary cortisol responses to psychological stress ($p=0.47$) and plasma cortisol responses to dexamethasone suppression ($p=0.87$) and $ACTH_{1-24}$ stimulation ($p=0.62$) were not associated with exposure to famine in utero.
Conclusion: These findings may indicate that fetal programming of the autonomic nervous system is more important than the HPA-axis in linking prenatal exposure to famine to the increased susceptibility to cardiovascular and metabolic disease.

Disclosure: Was this work supported by industry? No.

D-07

The metabolic syndrome in adults prenatally exposed to the Dutch famine

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The metabolic syndrome in adults prenatally exposed to the Dutch famine.
Objective: The metabolic syndrome is a constellation of interrelated metabolic risk factors, which predisposes to the development of type 2 diabetes and cardiovascular disease. Studies of people who were small at birth have shown that the syndrome might originate from restricted intra-uterine growth. We aimed to determine whether exposure to famine in utero is associated with an increased prevalence of the metabolic syndrome at the age of 58.
Materials/methods: We measured metabolic and anthropometric variables and determined the prevalence of the metabolic syndrome as defined by NCEP in 783 members of the Dutch famine birth cohort. Cohort members were born as term singletons around the time of the 1944—1945 Dutch famine.
Results: Participants exposed to famine during late or early gestation more often had the metabolic syndrome, although

the difference compared to participants unexposed to famine during gestation did not achieve statistical significance (OR for late exposed = 1.4 [95% CI: 0.9 to 2.1], OR for early exposed = 1.4 [95% CI: 0.9 to 2.4], both adjusted for gender). Of the individual factors contributing to the metabolic syndrome only decreased HDL (<1.03 mmol/l for men and <1.3 mmol/l for women) occurred more often (OR for late exposed = 2.0 [95% CI: 1.2 to 3.2], OR for early exposed = 2.1 [95% CI: 1.2 to 3.8]). **Conclusion:** Prenatal exposure to famine during late and early gestation seems to be related to a higher prevalence of the metabolic syndrome.

Disclosure: Was this work supported by industry? No.

D-08

Anthropometry in middle age following exposure to famine during gestation: evidence from the Dutch famine

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Background: Few studies in humans have related maternal undernutrition to offspring adult body size. We used the circumstances of the Dutch Famine of 1944—1945 to assess whether generalized reductions of maternal food intakes during pregnancy were related to offspring weight and tissue distribution in middle age.
Methods: We recruited (1) exposed individuals born in one of 3 institutions in western Holland between January 1945 and March 1946, whose mothers experienced famine during or immediately preceding pregnancy; (2) unexposed individuals born in the same three institutions during 1943 or 1947, whose mothers did not experience famine during this pregnancy; and (3) unexposed same-sex siblings of subjects in series 1 or 2. Anthropometric measurements (n = 436 M, 534 F) were obtained between 2003 and 2005. We defined four (partially overlapping) windows of gestational exposure (by ordinal weeks 1—10, 11—20, 21—30, and 31 through delivery) based on exposure to a western-Holland ration <900 kcal/day during the whole 10-week interval.
Results: Exposure to reduced rations was associated with increased weight and fat deposition at several tissue sites in women but not men (test for interaction p < 0.01). Measures of length and body proportion were not associated with famine exposure.
Conclusions: Reduced rations in mid-gestation were associated with increased female adiposity later in life.

Disclosure: Was this work supported by industry? No.

E. CANCER

E-01

The effect of the 1944—1945 Dutch famine on breast cancer risk: exploration of the national cancer registration

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Objectives: Explore the impact of the 1944—45 Dutch-Famine on breast cancer risk with data of the National Cancer Registration 1989—2003.
Materials/methods: Data on primary breast-cancers of women born 1927—1950 by province of residence allowed to estimate incidence-rates from number of life-born girls by birth cohort. The data allowed a geographical Famine exposure classification by grouping provinces according to their known exposure levels during the famine.
Results: Exposure at age 0.5—5 years indicated higher risks, with lower risks for age 9—14. When exposed in-utero, lower incidences were seen during first trimester and a higher incidence for second and/or third trimester of pregnancies. There were even indications for pre-conceptional/trans-generational effects.
Exposure 0—3 months before conception pointed to a lower incidence while exposure 3—6 months before conception pointed to higher incidences. Exposure longer than 6 months before conception showed no effects.
Conclusions: These results corroborate results from smaller size studies.
Given the likely (random) misclassification of exposure at province level, the effects found may reflect an underestimation of the impact of famine exposure.
New are indications for pre-conceptual/trans-generational effects as predicted by a set-point hypothesis. Results so far would predict that with a more valid famine exposure classification, e.g. by contrasting large heavily exposed cities vs. non-exposed cities, even when based on smaller numbers, effects of famine exposure would show up more clearly. Inclusion of other tumors, also among males, should allow to draw a more precise picture of the long-term impact of exposure to the 1944—1945 Dutch famine on cancer risk.

Disclosure: Was this work supported by industry? No.

E-02

The 1944—1945 Dutch famine and breast cancer risk

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Objectives: Childhood circumstances, including energy intake, may relate to later breast cancer risk. We explored whether the 1944—1945 Dutch famine is related to breast cancer risk with special interest in age at exposure.
Methods: We used data of a cohort of close to 15,000 women who were aged between 2 and 33 years during the 1944—1945 Dutch famine. Between 1983 and 1986, these women were asked about their famine experiences (absent, moderate, or severe exposure). During follow-up until January 2000, 585 new breast cancer cases were identified by the regional cancer registry. The relation between famine and breast cancer risk was assessed by weighted Cox regression models, in which a 15% random

sample was used to represent person-years lived in the entire cohort. Adjustments were made to account for potential confounders.
Results: The risk of breast cancer was significantly higher in women having been severely exposed to famine compared to women having been unexposed (hazard ratio (HR): 1.48 (95% confidence interval (CI): 1.09—2.01)). Risk in women having been moderately exposed also tended to be higher (HR: 1.13 (95% CI: 0.92—1.38), *p* for trend: 0.02). The relation tends to be stronger for women having been exposed before the age of 10 years (severely vs. unexposed: HR: 2.01 (95% CI: 0.92—4.41)). Exposure in adulthood was not associated with breast cancer risk.
Conclusions: Breast cancer risk is increased in women who were severely exposed to a short but severe famine decades earlier, especially if exposure was before 10 years of age.

Disclosure: Was this work supported by industry? No.

E-03
Prenatal smoke exposure and mammographic density

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Objective: The effect of passive smoke in adulthood has been examined with respect to breast cancer risk. Little is known, however, about whether passive smoke exposure earlier in life, in particular during the intrauterine period, may impact future breast cancer risk and whether this association, if it exists, is mediated by birth weight. We undertook a study of prenatal smoke exposure and mammographic density, a strong indicator of future breast cancer risk.
Methods: We contacted former female participants of the New York site of the U.S. National Collaborative Perinatal Project who were born between 1959 and 1966. Information on maternal smoke exposure was collected prospectively and the cohort followed until 7 years. To date, we have successfully contacted and obtained epidemiologic data on 262 women. 188 women (72%) have had a mammogram and 76% consented to share copies. We evaluated density using Cumulus software and estimated the associations using multivariate linear regression.
Results: 38% of the women had prenatal smoke exposure. Maternal smoking was negatively associated with breast density although the association was not statistically significant (−2.3, 95% CI: −8.8 to 4.0). The relationship did not change materially when adjusted for adult body size at age 20. The association was not mediated by birth weight or early childhood growth.
Conclusions: Overall we observed a modest negative association between prenatal smoke exposure and breast density. Although this association was not statistically significant, it was consistent with the few studies to date assessing this relation.

Disclosure: Was this work supported by industry? No.

E-04
Are cases of testicular cancer members of families with reduced fertility?

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Objective: Testicular dysgenesis syndrome (TDS) comprises testicular cancer (TC), hypospadias, cryptorchidism and poor semen quality. This syndrome is thought to have increased in prevalence in most western countries over the past decades, and environmental factors acting in utero are implicated in the etiology of TDS. Signs of reduced fertility in those who were later to be diagnosed with TC, include fathering a smaller number of children, a reduced sperm count, a lower proportion of boys among offspring, and reduced dizygotic twinning rate. The aim of this study was to obtain an indication of whether reduced fertility is a trait being present already among the parents of the TC cases.
Materials and methods: Census data from Statistics Norway were linked with data from the Cancer Registry of Norway. A case-control study was undertaken to compare the fertility among the parents of 3743 TC cases and 374,300 controls, matched by year of birth.
Results: The number of children was reduced among the parents of the TC cases, assessed by odds ratio (OR), among both their mothers [OR=0.95, *p* (trend) <0.01] and fathers [OR=0.97, *p* (trend) <0.06]. The proportion of unlike-sex twins (as a proxy for dizygotic twinning rate) was reduced among both their mothers (OR=0.56, $p<0.05$) and fathers (OR=0.55, $p<0.05$). ORs were adjusted for the respective parent's age.
Conclusion: These signs of reduced fertility among the parents of the TC cases, indicate that the genetic component prevails at the expense of the environmental component in the association between subfertility and TC, and thus TDS.

Disclosure: Was this work supported by industry? No.

E-05
The influence of caloric restriction due to the Dutch famine on breast density; a study in participants of the DOM breast cancer screening cohort

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Objective: Long-term caloric restriction is known to reduce cancer risk in animals. Recently, we showed that short, intense caloric restriction due to the Dutch famine increased breast cancer risk in women and had an effect on the gonadal hormone and IGF-system. These systems also affect breast density. Breast density has consistently been established as one of the strongest risk factors for breast cancer in women. We set out to determine the influence of

the caloric restriction on density of the parenchymal pattern.
Materials/methods: The mammograms of a random sample of 1035 women were measured as to dense and non-dense tissue and the relative density in a continuous scale. The results were adjusted for known determinants and related to the level of exposure to the famine in all women and strata of age at exposure.
Results: There was no overall relation between famine exposure and breast size (124 to 121 cm^2, *p*-trend = 0.50), amount of dense or non-dense tissue (respectively 23.4 to 21.8 cm^2, *p*-trend = 0.48 and 87.7 to 85.4 cm^2, *p*-trend = 0.55), or relative density (22.8% to 22.3%, *p*-trend = 0.78). When analysed according to age at exposure, non-dense tissue was significantly lower in women severely exposed before age 10, compared to unexposed women (53.1 cm^2 vs. 77.8 cm^2, *p*-trend = 0.03). Although not statistically significant, this group also appeared to have smaller breast with more absolute and relative density.
Conclusion: A short, intense caloric restriction has no overall effect on breast tissue in women, but may have some effect in women exposed before puberty.

Disclosure: Was this work supported by industry? No.

E-06
The risk of testicular cancer in Danish immigrants and their offspring

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Objective: To investigate the impact of immigrating to Denmark on the risk of testicular cancer (TC). Differences in risk of TC in the immigrant population according to country of origin, duration of stay, and age at immigration were calculated, and the risk of TC in the immigrant population was compared with the risk among their offspring born in Denmark.
Materials and methods: Danish population registers were used to identify the study population. Information concerning country of origin, history of immigration, emigration, and TC incidence was obtained on 2,212,696 persons representing 45,602,052 person-years of follow-up and 4226 TC cases. Estimation of relative risk (RR) was based on a log-linear Poisson regression.
Results: The risk of testicular cancer in the immigrant population was not modified by age at immigration or duration of stay in Denmark. Offspring born in Denmark by two immigrant parents had a RR of 2.24 (1.17, 4.27) compared with first generation immigrants.
Conclusion: The immigrant population preserved their country of origin's risk of TC independently of age at immigration and duration of stay in Denmark, reflecting the importance of genetic composition. The risk of TC in the second generation of immigrants was doubled compared with the first generation. Differences in genetic susceptibility cannot explain a doubling of risk in the second generation of immigrant. Thus an environmental factor present in Denmark and acting in very early life, perhaps in utero, appears to be responsible.

Disclosure: Was this work supported by industry? No.

E-07
Developmental aspects of cancer; intra-individual differences of cancer locations in paired organs and the developmental processes that they reflect

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Background: Comparing paired "identical twin" organs is a strategy to zoom in on differences in developmental trajectories, since intra-individual paired organs do not vary in age, genes, exposure to dietary, reproductive lifestyle, or other classical cancer risk factors which tend to vary between individuals. Patterns in observed differences—if existing—could eliminate several competing development-based hypothesis.
Design: Comparisons of left/right tumour ratios in the 1989—2003 National Netherlands Cancer Registry.
Results: Male breast left/right ratio 1.20 ($CI_{95\%}$ 1.05—1.37), female 1.08 ($CI_{95\%}$ 1.06—1.09). Other left/right differences were observed in paired endocrine organs: ovaries 0.97 ($CI_{95\%}$ 0.93—1.01), testis 0.90 ($CI_{95\%}$ 0.86—0.95), adrenals 1.17 ($CI_{95\%}$ 0.99—1.38), and the brain 0.98 ($CI_{95\%}$ 0.94—1.03) but not in kidneys 1.00 ($CI_{95\%}$ 0.97—1.03).
Discussion: (1) Male/female breast-cancer results point towards early development, unrelated to female reproductive factors. (2) Lack of inverse laterality of brain vs. (all) other tumours defies a simple neuronal innervation hypothesis, e.g. related to handedness. (3) A simple hormonal hypothesis seems unlikely. (4) A (cardio-)vascular perfusion hypothesis cannot explain these relevant differences. (5) A somite-specific development bound/organ size model remains, although an asymmetry in early cell division/mitochondria-number-imbalance-based hypothesis is also compatible with observed results. The left/right pattern with (endocrine) organs and/or body size could fit previous hypotheses by deWaard and Albanes on the role of the amounts of vulnerable target tissue on cancer risk. This INTRA-individual approach to study developmental origins of cancer shows a specific potential compared to a traditional INTER-individual approach.

Disclosure: Was this work supported by industry? No.

E-08
Early programming of lymphocytes, eosinophiles, and platelets after exposure to the Dutch famine; a study in the DOM breast cancer screening cohorts

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Background: Diabetes, cardiovascular diseases, and cancer are increased after severe exposure to the Dutch famine. In rats caloric restriction decreases thymus-derived cells though no relations have yet been found/studied addressing the human immune system.

Materials: Female participants (born 1932—1941, non/exposed 2592 and severely exposed 1031) provided in 1984—1986 information on famine exposure and donated blood between 1993 and 1997 that was analysed by Coulter-MaxR. This allowed to explore famine exposure at ages 3 to 12 on blood parameters measured 50 years later.
Results: Severely exposed women had higher white blood counts (6.40 vs. 6.24, $p=0.02$, R^2 4%) due to more lymphocytes (1.88 vs. 1.82, $p=0.01$) and eosinophiles (0.15 vs. 0.14, $p=0.003$). Also higher levels of platelets were found (255.7 vs. 250.7, $p=0.04$) but no effects on erythrocytes were found.
Conclusion—discussion: These results seem the first to point out long-term programming effects on human blood cells, including platelets, as related to caloric restriction early in life. The effects are limited to two cell groups: (1) cells devoted to immune responses, of either bacterial (lympocytes) or parasitic (eosinophiles) infections and related inflammations, and (2) megakaryocyte derived platelets, involved in thrombus formation. This may reflect additional effects of caloric restriction in humans, representing new pathways, namely inflammation and thrombus formation, that could mediate effects of caloric deprivation on both the occurrence of chronic diseases and ageing. Infection related inflammatory effects would fit mechanisms proposed by C. Finch (2004, 2005) to explain ageing and/or reflect an immune set-point mechanism as previously proposed for hormones (vanNoord, 2004).

Disclosure: Was this work supported by industry? No.

E-09
Handedness and breast cancer; an exploration of early programming factors in the DOM breast cancer screening cohorts

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Background: A neuro-developmental hypothesis relates handedness to early brain sex-steroids levels, originating (in)directly from either the mother or the foetal/placental unit. A previous study in this cohort confirmed increases of breast cancer risk in left-handed women. Handedness itself showed no relation to the left or right sided tumour occurrence.
Methods: This breast-cancer screening cohort allowed additional analysis of relations with handedness among women without breast cancer; link adult blood estrogen levels in $n=196$ participants in an osteoporosis sub-study. Relations with mammographic density (dysplasia yes/no) among $n=8.763$ women.
Results: Higher E1 concentrations were seen in right-handed women ($p=0,09$). A relation of left-handedness with Dy showed a crude OR 1.04 ($CI_{95\%}$ 1.00—1.07), while adjusted for BMI and number of children 1.13 ($CI_{95\%}$ 0.99—1.30).
Conclusions: Observed difference of E1 levels would fit above handedness hypothesis and may provide a biological explanation for a possible link to breast cancer risk. Relations with Dy seem to oppose observed associations with breast cancer risk. Handedness may mark life-long differences in set-points of gonado-trophines and/or sex-steroids levels. The results indicate a dissociation between (intermediate) risk factors E1 and Dy and observed average relations with breast cancer. This could indicate that inferences on population-based, "mean" level risk-factors have limited validity to explain cancer occurrence at lower—let alone individual—levels of aggregation. Studies taking into account relations conditional on where they occur on a developmental time axis may come closer to explain relations with relevance for interventions.

Disclosure: Was this work supported by industry? No.

E-10
Associations of birth weight and maternal history of miscarriage with childhood cancers

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Objective: Higher birth weight and maternal history of miscarriage have been associated with an increased risk of childhood cancers, but have not been investigated together previously. Possible interactions between these factors and sex on disease occurrence were investigated.
Methods: In a retrospective case-control study, 732 childhood (= 15 years) cancer cases from the Northern Region Young Persons' Malignant Disease Registry in Northern England whose hospital birth records could be accessed and 3723 controls matched for date and hospital of birth to the cases were compared. Conditional logistic regression was used to analyse the data.
Results: Maternal history of miscarriage showed an association with all cancers and individually with acute lymphoblastic leukaemia (ALL) and neuroblastic tumours. The miscarriage association with ALL was statistically significant in boys only (OR = 1.91, 95% CI = 1.07 to 3.42). Birth weight and sex showed a strong interaction ($p=0.002$). In boys with ALL, but not in girls, there was a nonlinear association with birth weight (OR = 3.76 for the highest quintile, 95% CI = 1.62 to 8.73). A multivariable model for ALL confirmed the independence of associations with miscarriage history and birth weight.
Conclusions: The findings suggest that size at birth, rather than weight for gestational age, may be of aetiologic importance in childhood ALL. Statistically significant associations of size at birth and maternal reproductive history with childhood cancer suggested marked differences in etiology between girls and boys.

Disclosure: Was this work supported by industry? No.

E-11
Birth weight as a predictor of breast tissue density

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Objective: Birth weight has been positively associated with breast cancer risk. However the limited research into the relationship between birth weight and breast tissue density has not found an association. The potential relationship between birth weight and breast tissue density was investigated using data from the Newcastle Thousand Families Study (NTFS).
Methods: The NTFS was initiated in 1947 when all 1142 babies born in May and June that year to mothers resident in the city of Newcastle were recruited into the study. During childhood, detailed information was collected prospectively on factors including birth weight and social class. At age 50, 574 study members returned a self-completion questionnaire and 412 attended for clinical examination. The 307 surviving women who returned questionnaires at age 50 were sent a questionnaire asking for details of mammographic screening. Wolfe grades were assigned to breast tissue patterns and breast size measured. Ordinal logistic regression was used in the analysis.
Results: Questionnaires were returned by 236 women, with 203 initial mammography films available. The distribution of Wolfe grades was follows: N1 (lowest risk of breast cancer) 22, P1 39, P2 97, and DY (highest risk) 44. Birth weight, standardized for gestational age and gender, was positively associated with increased Wolfe grade (ordinal OR 1.27, 95% CI 1.00—1.61, $p = 0.05$).
Conclusions: The finding of a significant positive association between birth weight and breast tissue density is consistent with previous research suggesting that heavier babies have an increased risk of breast cancer in later life.

Disclosure: Was this work supported by industry? No.

E-12
Birth size and subsequent breast cancer risk: a pooled analyses of individual data on over 20,000 breast cancer cases from 28 studies

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Objectives: Several studies have raised the possibility that prenatal environment, for which birth size is a proxy, may be a marker of subsequent breast cancer risk but their findings have been inconsistent. An international collaborative group was set up to re-analyse individual data from published and unpublished studies to assess possible sources of heterogeneity and to obtain more precise effect estimates.
Methods: We obtained individual data on birth size and breast cancer risk from 28 published and unpublished studies, comprising over 20,000 breast cancer cases. Random effect models were used to assess heterogeneity and, if appropriate, to combine study-specific estimates of effect.
Results: Preliminary analyses revealed a positive association between birth weight and breast cancer risk for studies based on birth or school records (pooled relative risk (RR) for 500 g increase in birth weight 1.06, 95% CI 1.02—1.10), but not for those based on maternal or self-recall (RR = 0.98 (0.92—1.05) (p for heterogeneity between sources of birth weight data = 0.03)) probably because the latter are associated with higher levels of exposure misclassification. Birth length and head circumference, available only from birth records, were also positively associated with breast cancer risk. The birth size effect persisted after adjustment for gestational age, anthropometric and socioeconomic measures in childhood or adulthood, and reproductive-related factors, and was not modified by age and/or menopausal status.
Conclusions: These findings are consistent with birth size being a marker of breast cancer risk later in life, with its effect being largely independent of known postnatal risk factors.

Disclosure: Was this work supported by industry? No.

E-13
Implications of sibling design: a life course study of fetal growth and breast cancer risk

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Objective: Recent epidemiologic studies suggest an association between markers of fetal growth and breast cancer risk later in life. But most fail to adequately control for confounding by family factors. We are conducting an adult follow-up study of a prospective birth cohort using a sibling sample to examine the relationship between pre- and postnatal factors and mammographic density, a strong indicator of future breast cancer risk.
Methods: Our study builds upon the Child Health and Development Study (CHDS) which collected prospective information from close to 20,000 mothers and their offspring born from 1959 to 1967. Extensive information exists from questionnaires, maternal medical chart information, neonatal, and childhood clinic visits. In addition, maternal sera during pregnancy was collected and stored. In this study of breast cancer risk, we are tracing and collecting adult epidemiologic assessments for all female siblings eligible for adult follow-up.
Results: Mothers of siblings eligible for our sample were similar in family-level factors of race/ethnicity, education, and income then maternal behavioral factors such as cigarette smoking compared to the entire cohort. Mothers of eligible siblings were younger and more likely to be married at enrollment.
Conclusion: The sibling sampling design has methodological advantages in life course studies and allows the implementation of unique tracing strategies to locate subjects. Inclusion criteria induced minimal selection bias on family level demographic variables.

Disclosure: Was this work supported by industry? No.

E-14
The influence of maternal weight gain, birth weight, and postnatal childhood growth on mammographic density

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Objective: Mammographic density is an important intermediate marker for breast cancer risk both because of its strength of association (4—6-fold) and the similar relations with most adult breast cancer risk factors. However, much of the variation in breast density is unexplained by adult risk factors. We undertook a study to understand whether pre- and postnatal factors are associated with mammographic density later in life.
Methods: We contacted former female participants of the New York site of the U.S. National Collaborative Perinatal Project who were born between 1959 and 1966 and prospectively followed for 7 years. To date, we have successfully contacted and obtained epidemiologic data on 262 women. 188 women (72%) have had a mammogram and 76% consented to share copies. We evaluated density using Cumulus software and estimated the associations using multivariate linear regression.
Results: Increased maternal weight gain, higher birth weight, rapid growth from birth to 4 months, and rapid growth from 1 to 7 years were all statistically significantly associated with lower breast density, even after adjusting for adult body mass index ($R^2 = 0.33$).
Conclusion: These data, if replicated, suggest that the positive relation between birth weight and other markers of fetal growth and breast cancer risk do not work through increasing mammographic density. The association between birth weight and breast cancer may work through another pathway, and its overall association may be even stronger after considering the negative relation between birth weight and growth and mammographic density.

Disclosure: Was this work supported by industry? No.

E-15
Incidence and survival characteristics of malignant diseases among holocaust survivors that have immigrated to Israel

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Background: Israelis of European and American origin exhibit high cancer morbidity. Many of them were extensively exposed to severe conditions during World War II (WWII) in Europe. Former studies in non-Jewish Dutch subjects, exposed to extreme famine during WWII, disclosed higher risk of breast and prostate cancer.
Aim: to assess cancer incidence and survival in Jewish holocaust survivors.
Methods: A prospective historical cohort based on Jews born in Europe who immigrated to Israel before (non-exposed) and after (exposed) WWII. Cancer morbidity and survival data were obtained through linkage with the National Cancer Registry and the Central Bureau of Statistics, respectively. Standardized incidence Ratios (SIRs) and 95% confidence intervals (95% CI) were calculated for total and specific cancer sites.
Results: The exposed and non-exposed groups contributed 4,011,264 and 908,436 person-years of follow-up, with 45,206 and 10,282 cancer cases, respectively. SIRs for all cancer sites in the exposed group ranged 1.14 to 3.42 and were statistically significant in both genders. Highest risks were observed in the youngest birth cohort: SIR (95% CI) were 3.42 (3.23, 3.61) and 2.32 (2.22, 2.41) for males and females born in 1940—1945, respectively. This pattern was especially pronounced for breast and colorectal cancer. Five years survival rates were lower in the exposed vs. the non-exposed by 5—13%, and holocaust survivors were often diagnosed at more advanced disease stages.
Conclusions: Holocaust survivors present higher cancer incidence, especially breast and colon cancer, and lower survival rates compared to immigrants not exposed to WWII. Those exposed at younger ages were mostly affected.

Disclosure: Was this work supported by industry? No.

F. STRESS, NEUROBEHAVIOUR, NEURODEVELOPMENTAL OUTCOME

F-01
Associations between midwife presence during labour, intrapartum interventions, and outcomes

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Events during labour may have an effect on the outcome of birth. So far the presence of midwives which may be interrelating with intrapartum interventions has not been studied.
Objective: The global hypothesis that the dynamics of labor are more influenced by time-dependent factors (like intrapartum interventions) than by pre-existing ones (e.g. education—however not parity) is examined with respect to the actual presence of the midwife to the labouring women.
Methods: The analyses are based on a sub-sample of 550 primigravid and multigravid women of a state-wide observational study on dynamics of labour who were at least 37 weeks pregnant, had no risk factors, and a spontaneous delivery. Their courses of labour and midwifery presence were exactly documented. Pre-existing and time-dependent factors are examined and their associations and correlations analyzed by exploratory and transitional data analysis (TDA).
Results: There are no significant associations between midwife presence and pre-existing factors (e.g. education) nor non-medical (e.g. immersion), or medical interventions (e.g. epidural). However, there is an association between duration of midwife presence (as per duration of birth) and APGAR scores at 1 min after birth. Transitional data analysis (TDA) with time-dependent variables follows.
Conclusion: Midwife presence is independent of pre-existing factors like education, and non-medical or medical interventions. This means that the absence of medical or non-medical interventions is not associated with a decrease in the presence of midwives. Longer midwife presence per duration of birth relates to better APGAR scores.

Disclosure: Was this work supported by industry? No.

F-02

The effects of maternal stress during different trimesters of pregnancy on problem behavior of 18—48-month-old children

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Objectives: Recent studies show stress and emotional complaints during pregnancy to be associated with negative consequences for infant development (Van den Bergh et al., 2005). However, little is known about critical trimesters during pregnancy for the association between prenatal stress and child outcomes. Moreover, when mothers are currently suffering from emotional complaints, they may rate the behavior of their children more negatively (Mednick et al., 1996). Therefore, fathers of the children are also included and asked to rate their children's development.
Methods: A sample of healthy pregnant women ($N = 500$) completed the Edinburgh Depression Scale (EDS) and the State-Trait Anxiety Inventory (STAI) in weeks 12, 24, and 36 of pregnancy. When the children were 18—48 months of age these mothers completed the EDS again and the Child Behavior Check List to assess behavior problems of their children.
Results: Preliminary results (147 children) show correlations between EDS scores in weeks 12, 24, and 36 and CBCL scores, especially externalizing problems of 0.26, 0.23, and 0.24, respectively. Correlations between STAI and CBCL for externalizing problems were respectively 0.31, 0.27, and 0.34. The externalizing behavior problem score of mothers and fathers correlated 0.67. Correlations for the fathers' CBCL externalizing score and the mothers' EDS and STAI at 12, 24, and 36 weeks during pregnancy were 0.32, 0.22, and 0.21 respectively as well as 0.36, 0.37, and 0.35.
Conclusion: The final results will be discussed in terms of the effect of maternal stress in critical periods during pregnancy on children's later development.

Disclosure: Was this work supported by industry? No.

F-03

The impact of fetal growth on the adrenocortical stress response is gender-specific

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Objectives: It has been repeatedly demonstrated that size at birth is associated with stress responsivity in adulthood. In general, smaller size at birth has been shown to be related with a hyperactive hypothalamus pituitary adrenal axis, potentially caused by a central deficit of glucocorticoid receptors impairing negative feedback inhibition. While numerous animal studies only tested male subjects, studies implying both genders reported discrepant outcomes of fetal growth restriction in males and females.
Materials/methods: We compared the salivary cortisol response to a psychological stressor in male and female university students ($N = 44$, mean age: 23) born small respectively appropriate for gestational age.
Results: A pronounced gender effect was observed in the growth-restricted group, in terms of a high cortisol increase in the males in response to the stressor, while no increase was observed in the female subjects.
Conclusions: These results suggest that intrauterine growth restriction might impact on the adrenocortical stress response in a gender-specific way. The findings are discussed in the context of gender prevalence in stress-related disorders, characterized by a relative lack of cortisol respectively excessive endogenous cortisol exposure.

Disclosure: Was this work supported by industry? No.

F-04

Early postnatal growth and cardiovascular reactivity to psychological stressors in late adulthood

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Objective: Specific postnatal growth patterns strongly relate to risk of cardiovascular (CV) disease outcomes in later life, but the mechanisms of this link are unclear. We studied whether CV-reactivity, an antecedent for several CV-disease outcomes, is associated with postnatal growth.
Methods: 73 men and 80 women from Helsinki Birth Cohort, born 1934—1944, whose height and weight were repeatedly recorded during the first 10 years, underwent the Trier Social Stress Test (TSST). Beat-to-beat blood pressure was monitored via non-invasive finger photoplethysmography (Finometer®), and reactivity scores were determined as the mean increment from baseline value. Conditional method was used to characterize growth independent of earlier growth, for periods of 0—2 years, 2—7 years, and 7—10 years. Analyses were adjusted for age, current size, baseline CV-value, and the use of beta-blocker and depression medication.
Results: In women, cardiovascular reactivity was positively associated with BMI gain during 7—10 years. Systolic reactivity increased by 6.0 mm Hg (95% CI, 1.8 to 10.2) and diastolic reactivity by 2.1 mm Hg (95% CI, 0.1 to 4.1) for every standard deviation increase in BMI gain during this period. In men, height gain during 0—2 years was positively associated with diastolic reactivity ($B = 3.0$, 95% CI 0.9 to 5.1) and marginally associated with systolic ($B = 4.4$, 95% CI −0.1 to 9.0) and heart rate ($B = 3.2$, 95% CI 0.0 to 6.4) reactivity.
Conclusions: The associations between postnatal growth and CV-reactivity could explain part of the links observed between postnatal growth and cardiovascular diseases.

Disclosure: Was this work supported by industry? No.

F-05
Antenatal maternal stress/anxiety and effects on child neurodevelopment

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There is good evidence, from independent prospective studies, including our own, that if the mother is anxious/stressed while pregnant, her child is substantially more likely to have behavioural, emotional, or cognitive problems. An increased risk of attention deficit/hyperactivity, anxiety, and language delay have been described. We have found that cortisol levels are raised in her 10-year-old child. These effects are independent of those due to postnatal depression and anxiety. We still do not know what forms of anxiety or stress are most detrimental, but our recent research suggests that the relationship with the partner can be important in this respect.
These effects are presumably due to epigenetic mechanisms, such as a change in the methylation status of the gene for the glucocorticoid receptor. The fetal environment can be altered if stress in the mother changes her hormonal profile; we have shown is a strong correlation between maternal and fetal cortisol levels. However, many problems remain in understanding mechanisms, and even the role of the HPA axis. The mother's cortisol responses to stress become very much reduced at the end of pregnancy. In earlier pregnancy the link between maternal and fetal cortisol is less robust. It has been suggested that the evolutionary function of fetal programming has been to prepare the child for the particular environment in which he/she is going to find themselves, the predictive adaptive response. A hyper-responsive HPA axis, extra vigilance or anxiety, or readily distracted attention may have been adaptive in a stressful environment.

Disclosure: Was this work supported by industry? No.

F-06
Critical periods of fetal and early childhood brain growth predict childhood cognitive function

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Objective: To determine if critical periods of neurodevelopment which affect intelligence quotient (IQ) occur in fetal and early childhood life.
Methods: Serial prenatal ultrasounds were performed on 1349 high-risk pregnant Scandinavian women followed for 5 years in the NICHD Study of Successive Small for Gestational Age Births. *Z*-scores were calculated for fetal biparietal diameter (BPD) growth velocity and postnatal head circumference (HC) growth velocity up to 5 years. Brain growth velocities were used to predict IQ score at 5 years, while controlling for potentially confounding socioeconomic factors.
Results: In a multivariate linear regression model predicting total IQ score at 5 years, *z*-scores for in-utero BPD growth and postnatal HC growth, gender, and several socioeconomic measures were included as independent predictors. BPD growth in the first trimester was inversely associated with IQ ($\beta = -4.07$, $p < 0.001$). BPD growth in the early third trimester and HC growth between 1 and 5 years demonstrated trends towards significance in predicting IQ scores ($\beta = 1.68$, $p = 0.06$ and $\beta = 2.39$, $p = 0.05$). Other significant predictors included gender ($\beta = 4.54$, $p = 0.006$), maternal age ($\beta = 0.66$, $p = 0.002$), and maternal education ($\beta = 3.89$, $p < 0.001$). Paternal age, paternal education, smoking, and duration of breastfeeding were not significant.
Conclusions: These results suggest that fetal brain growth during the first trimester has a strong inverse relationship with childhood IQ.

Disclosure: Was this work supported by industry? No.

F-07
Length at birth predicts hypothalamic—pituitary—adrenal axis (HPAA) responsiveness to psychosocial stress at age 60

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Objective: We studied whether body size at birth predicts HPAA response to psychosocial stress, which is a possible mechanism linking early life conditions with adult disease.
Methods: 285 members of the Helsinki Birth Cohort (141 men and 144 women), born between 1934 and 1944, underwent the Trier Social Stress Test (TSST), with salivary and plasma ($n = 215$) samples obtained at baseline and at 1—10—20—30—45—60—90 min after the TSST. Cortisol was measured from all and ACTH from the four first plasma samples. All concentrations were log-transformed and analyses were adjusted for sex, current age, body mass index, gestational age, and time of the TSST (10.30, 12.15, or 14.00, dummy coded).
Results: Mean salivary cortisol concentrations rose from 7.1 μg/l at baseline to a maximum of 14.7 μg/l after stress. Plasma cortisol rose from 92.5 to 166.0 μg/l and ACTH from 20.8 to 48.4 μg/l. The rise was unrelated to gestational age or body size at birth. However, length at birth predicted plasma cortisol and ACTH concentrations: one cm increase in length at birth was associated with 0.07 S.D. (0.00 to 0.14) higher cortisol area-under-the-curve (AUC), 4.5% (0.6 to 8.4%) higher ACTH at baseline and 7.9% (3.1 to 12.9%) after stress, 0.11 S.D. (0.05 to 0.18) higher ACTH AUC, and 0.11 S.D. (0.04 to 0.18) lower ratio of cortisol AUC to ACTH AUC. No relationship was seen with salivary cortisol. Findings were similar in both sexes.
Conclusions: Short length at birth predicts lower ACTH and cortisol concentrations during psychosocial stress and lower responsiveness of the adrenal cortex to endogenous ACTH. The finding reinforces suggestions that HPAA responsiveness to psychosocial stress is programmed during early life.

Disclosure: Was this work supported by industry? No.

F-08

Lower number of sex partners in young adults born with very low birth weight

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Objective: Little is known about the effects of perinatal conditions on reproductive function in humans. We studied the sexual history of young adults born severely preterm, who serve as a model of extreme adverse perinatal conditions.
Methods: The subjects were young adults (18 to 27 years) born with very low birth weight (VLBW, <1500 g) and controls born at term (≥37 weeks' gestation) matched for sex, age, and birth hospital. 185 VLBW-born subjects (105 women and 80 men) and 196 controls (117 women and 79 men) filled in a questionnaire including questions about sexual history. 25 subjects with a major disability were excluded. Mean birth weight was 1128 g (S.D. 216) in the VLBW and 3591 g (483) in the control group; gestational ages were 29.2 (2.4) and 40.1 (1.3) and birth weight S.D. scores − 1.2 (1.6) and 0.0 (1.0).
Results: Young adults born with VLBW reported a smaller number of lifetime sex partners (χ^2, $p=0.01$ for women and 0.08 for men; p for linear trend=0.003 and 0.03, respectively)

		Number of sex partners			All
		0	1 to 3	≥4	
Women	VLBW	23	27	43	93
	Term	15	24	77	116
Men	VLBW	22	20	26	68
	Term	13	25	40	78

Among females, VLBW birth was associated with later age at first sexual intercourse (Cox regression $p=0.02$). This difference was not seen among males ($p=0.2$). Adjustment for height, BMI, or age had little effect on the results.
Conclusions: Severely preterm birth may be associated with altered reproductive performance in adult life.

Disclosure: Was this work supported by industry? No.

F-09

Fetal programming of big five-personality traits: a longitudinal follow-up of 60 years

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Objective(s): Personality traits are associated with health-related risks in adulthood. Many studies have shown that prenatal factors predict subsequent cognitive, behavioral, and health-related outcomes, but very few have examined whether neonatal characteristics associate with adult personality traits. We tested whether gestational age or body size at birth predict personality traits in late adulthood.
Material/method(s): Participants were 1702 men and women born at or over 37 weeks of gestation in Helsinki between 1934 and 1944 (Helsinki cohort) who attended follow-up survey in 2004 and fulfilled the NEO Personality Inventory (NEO-PI). We tested with linear regression analyses whether gestational age and body size at birth, as continuous, or categorized variables, predicted personality traits neuroticism, extraversion, agreeableness, consciousness, or openness to experience at the age of 63.4 years.
Result(s): Longer gestational age was associated with higher consciousness after controlling for sex, age, and birth weight ($p<0.05$). Participants belonging to the smallest group with any of the body size measures at birth (weight ≤2.5 kg, length <48 cm, head circumference <33 cm) scored higher on neuroticism (p's<0.05 for weight and length; $p=.053$ for head circumference) with adjustments for gender and gestational age. Adjustment for childhood and adulthood socioeconomic status, age, and BMI had little effect on the results.
Conclusion(s): Gestational age and birth size predicted NEO-PI personality traits in late adulthood. Longer gestational age predicted higher consciousness and smaller birth size predicted higher neuroticism. These personality traits, in turn, are associated with health and mortality in late adulthood.

Disclosure: Was this work supported by industry? No.

F-10

Do prenatal factors predict our temperaments? 60 to 70 years follow-up study in the Helsinki cohort

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Objective(s): Prenatal factors predict various cognitive, behavioral, and health-related outcomes later in life. Although adult temperamental traits are known to modulate risk for several psychiatric disorders, few studies have examined whether newborn characteristics associate with temperament in adulthood. We tested whether gestational age and body size at birth predict temperament in late adulthood.
Material/method(s): Participants were 1527 men and women born at or over 37 weeks of gestation in Helsinki between 1934 and 1944 (Helsinki cohort) who attended follow-up survey in 2004 and fulfilled 98-item Cloninger's Tridimensional Personality Questionnaire (TPQ). We tested with linear regression analyses whether body size at birth and gestational age as continuous or categorized variables predict temperamental traits of novelty seeking (NS), harm avoidance (HA), or reward dependence (RD) at the age of 63.4 years.
Result(s): Weight, length or head circumference at birth, or gestational age were not linearly associated with any of the TPQ dimensions (p's>0.11). However, after adjusting for

gender and gestational age, those participants belonging to the smallest group in weight (≤ 2.5 kg), length (<48 cm), or head circumference (<33 cm) at birth scored higher on HA (p's<0.05) compared to others. These associations remained after further controls for age, and adult BMI and socioeconomic status.
Conclusion(s): Prenatal factors contribute to temperament even in late adulthood. Small body size at birth predicted higher HA, which in turn may predispose to psychiatric disorders such as depression.

Disclosure: Was this work supported by industry? No.

F-11
Effect of maternal stress on the fetal activity using the vibroacoustic stimulation and the four-dimensional (4D) ultrasound examination

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Objectives: Our purpose was to determine whether maternal state and trait anxiety levels affect fetal biophysical activity.
Materials/methods: Fifty healthy pregnant women with a singleton pregnancy were enrolled. Maternal anxiety was assessed using the Spielberger State-Trait Anxiety Inventory (STAI) at 20, 30, and 35 weeks of gestation. Women in the high anxiety groups had scores equal to or above the median. At 20 weeks of gestation, we observed fetal facial expression and hand to face movements using 4D ultrasound. 4D images were displayed on the screen and recorded on the videotape during the 30-min observation period. At 30 weeks of gestation, fetal heart rate (FHR) pattern before and after the vibroacoustic stimulation (VAS) was recorded.
Results: All neonates were born in satisfactory condition. The 1- and 5-min Apgar scores were greater than 7 and 9, respectively. The median state anxiety score was 39, and the median trait anxiety score was 40. These scores were not changed throughout the pregnancy. The frequency of mouth opening in the fetus in high trait anxiety group was significantly less than those in the low trait anxiety group (2.7 ± 2.4 vs. 8.2 ± 6.4, $p<0.05$), while other movements did not differ between the two groups. The FHR response after VAS in pregnant women with high trait anxiety showed poor response (8.6 ± 2.3 vs. 16.2 ± 6.5, $p<0.01$).
Conclusions: These findings suggest that stress in pregnant women with high anxiety may influence the fetal biophysical activity including FHR patterns after VAS.

Disclosure: Was this work supported by industry? No.

F-12
Antenatal maternal anxiety is associated with impairments in cognitive control: involvement of the orbitofrontal cortex

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Objective: The development of the fetus, child, and even adolescent is found to be negatively influenced by anxiety and stress experienced by the mother during pregnancy. Previously associations were found with emotional and behavioural development. Recently clear influences on cognitive development were found. The aim of the presented study was to extend the findings of the follow-up study of Van den Bergh et al. (2005, Neuroscience and Biobehavioral Reviews, 29, 259—269; 2006, Pediatric Research, 59, 78—82). These studies found specific cognitive impairments related to endogenous cognitive control in 14/15-year-old adolescents of mothers who experienced high levels of anxiety during their pregnancy.
Methods: Cognitive functioning of 49 17-year-old adolescents was assessed using five tasks typically linked to activations in different areas of prefrontal cortex. In the same sample event-related potentials (ERP) were used to investigate actual brain processes.
Results: Performance on a dual task and a response shifting task was found to be impaired in the high antenatal maternal anxiety group. Working memory, cued visual attention, and external response inhibition on the other hand were not related to antenatal maternal anxiety.
Conclusions: The present results corroborate and extend the findings of our previous follow-up stages. The specific cognitive impairments found could be linked to the orbitofrontal cortex. It is hypothesized that these impairments could be linked to subtle aberrations or fetal programming during the development of prefrontal cortical areas and networks.

Disclosure: Was this work supported by industry? No.

F-13
An early life profile of immune and sickness behaviours following prenatal endotoxin exposure

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Objectives: Exposure of the pregnant dam to bacterial endotoxin is an experimental model of prenatal bacterial infection in the human. This study aimed to characterize the effects that such exposure would have on immune and sickness behaviours in the neonate when exposed to an immune challenge.
Methods: Fischer 344 rats were exposed to saline or endotoxin (200 μg/kg, s.c.) during pregnancy (days 16, 18, and 20, term=23). On postnatal day 5, the offspring were exposed to an immune challenge (LPS, 50 μg/kg, i.p.) to assess sickness and immune responses. Pup weights were recorded and trunk blood was collected prior to or 4 h post-LPS for analysis of corticosterone, interleukin-1beta (IL-1b) and tumor necrosis factor alpha (TNFa) levels.
Results: At baseline, no differences were observed between the prenatal treatment groups in corticosterone, IL-1b or TNFa levels. While all offspring had elevations in corticosterone, IL-1b and TNFa levels above baseline at 4 h post-LPS administration, the offspring of endotoxin-treated mothers had significantly less corticosterone, TNFa and IL-

1b levels ($p<0.05$) than the controls. This was associated with a significant weight gain in the offspring of endotoxin-treated mothers, which was not apparent in the control offspring. This is indicative of differences in appetite suppression over the 4 h in the prenatal endotoxin group.
Conclusions: These results show evidence for altered immune and sickness behavioural outcomes following prenatal endotoxin treatment. Significantly, these differences are apparent in very early life, when bacterial exposure is common.

Disclosure: Was this work supported by industry? No.

F-14
Assessment of uterine circulation in a pregnant rat whose mother was fed a low protein diet

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Objective: Our previous study showed that a maternal low-protein diet induced hypertension and vascular dysfunction in rat offspring after day 175. In this study, we hypothesized that rat offspring that underwent maternal protein restriction showed hypertension in pregnancy even if they were younger than 175 days old because potential vascular dysfunction should become prominent in the load of pregnancy.
Methods: Wistar rats were fed a diet containing either 18% (group C) or 9% (group R) casein throughout pregnancy. The female offspring were mated between days 70 and 125. They were fed standard chow during pregnancy. Blood pressure was measured on day 19 or 20 of gestation. On day 20 or 21, the uterine arteries were dissected and their vascular function was investigated with a wire myograph.
Results: Weight gain during pregnancy was significantly smaller in the R group. There were no significant differences between two groups in systolic blood pressure. Vasoconstriction of uterine arteries to U46619 was increased in the R group, whereas vasodilatation to SNP was increased.
Conclusions: Offspring in the R group showed increased vasoconstriction for TXA_2 in their pregnancies. This alteration may be compensated by hypersensitivity for NO so as to keep the blood pressure normal in spite of the load of pregnancy. Maternal protein restriction lead to worsened uterine circulation in the offspring during pregnancy, and the pregnant offspring showed less weight gain. These results suggested that maternal undernutrition is likely to affect beyond generations.

Disclosure: Was this work supported by industry? No.

F-15
Hypothalamo—pituitary—adrenal axis responsiveness in adult sheep following early life nutrient restriction

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Objectives: Altered hypothalamo—pituitary—adrenal (HPA) axis activity is implicated in mediating the effects of the early life nutrient environment and metabolic and cardiovascular abnormalities in later life (Phillips 2006 J Physiol 572.1:45). We determined the long-term effects of early pre- and postnatal life nutrient restriction on adult HPA axis function in sheep.
Methods: Ewes received either 100% (C, $n=39$) or 50% nutritional requirements (U, $n=41$) from 1 to 31 days gestation and 100% thereafter. Male and female offspring were then fed either *ad libitum* (CC, $n=22$; UC, $n=19$) or to reduce body weight to 85% of target from 12 to 25 weeks postnatal age (CU, $n=17$; UU, $n=22$) and *ad libitum* thereafter. At age 1.5 and 25 years, ACTH and cortisol responses to CRF+AVP (0.5/0.1 μg/kg, i.v. bolus) were determined and analysed by ANOVA.
Results: In females only, at 1.5, but not 2.5 years, Δpeak ACTH responses were reduced ($p<0.05$) while Δpeak cortisol was maintained following postnatal undernutrition, regardless of prenatal nutrition. At both ages, cortisol (basal and stimulated) was greater ($p<0.05$) in females than males, with no difference in ACTH.
Conclusion: These findings suggest that undernutrition in early postnatal life decreases pituitary responsiveness to hypothalamic factors and enhances adrenal responsiveness to ACTH in adult sheep, in a sex- and age-specific manner.
Supported by BHF, BUPA and Hope.

Disclosure: Was this work supported by industry? No.

F-16
Individual differences in brain activation of mothers in response to infant cues

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Objective: The quality of the maternal environment influences the emotional well-being of the mother. Infant cues as well as the mother's emotional state are crucial for directing maternal responsiveness. Previous studies have described areas of increased brain activation associated with maternal responsivity; however, there is no evidence as to whether individual differences in the responses of mothers to their infants are associated with differential activation of these same brain regions.
Methods: Thirty-seven subjects (mean age: 29.78) were mothers recruited from an ongoing study (MAVAN). Maternal adversity and responsiveness was assessed through standardized questionnaire tests. We examined the neural correlates of maternal responsivity using functional magnetic resonance imaging (fMRI) in which mothers viewed pictures of their own infant and an unknown infant.
Results: Mothers with high levels of maternal adversity had significantly higher trait anxiety and higher cortisol levels than mothers with low levels of adversity. Analysis of the PBI revealed there was a significant negative correlation between mother care and mother overprotection and a negative correlation between trait anxiety scores and

mother care approached significance in the high maternal adversity group. We also demonstrated that mothers with high levels of maternal adversity had less activation within brain regions that are associated with maternal care in response to infant cues.
Conclusions: This is the first study that has investigated the interaction of maternal environment, emotional well-being, and brain activation in human mothers.

Disclosure: Was this work supported by industry? No.

F-17
Growth and neurodevelopmental outcome of very low birth weight infants delivered by pre-eclamptic mothers

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Objective: Evaluate growth and neurodevelopmental outcome at 1 year corrected age of VLBW infants delivered by pre-eclamptic mothers.
Methods: VLBW infants delivered between 11/2003 and 02/2005 in our institution followed up to 12 months corrected age were divided in maternal pre-eclamptic and non-pre-eclamptic groups. Exclusion criteria: death before 1 year corrected age, major malformations, deafness, and blindness. Birth weight, gestational age, adequacy to gestational age, presence of RDS, sepsis, BPD, PIVH, length of mechanical ventilation and hospital stay, and maternal education were compared between both groups. Weight, length, and head circumference were plotted on NCHS curves. Bayley scales at 1 year corrected age were compared between both groups.
Results: 65 VLBW infants: 35 in pre-eclamptic and 30 non-pre-eclamptic groups. There were no significant differences in studied perinatal factors, except for increased number of SGA in pre-eclamptic group ($p=0.01$). At 1 year corrected age, only head circumference had a trend to be decreased in pre-eclamptic group ($p=0.058$). None of the patients in both groups had MDI and PDI higher 85. Both groups had similar PDI. 64% of non-pre-eclamptic and 44% of pre-eclamptic had MDI lower than 80 ($p=0.091$).
Conclusion: Despite newborns of pre-eclamptic mothers have smaller head circumference, it does not reflect on their neurodevelopmental outcome at 1 year corrected age.

Disclosure: Was this work supported by industry? No.

F-18
Effects of prenatal stress exposure during World War II on psychological and medical factors in adulthood

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Objective(s): Prenatal stress has been found to profoundly alter brain stress circuits and, in consequence, might lead to an enhanced activity of the hypothalamic–pituitary–adrenal (HPA) axis in animals. Also in humans, first evidences emerge that prenatal stress exposure possibly affects central regulatory pathways and downstream hormone secretion. Furthermore, in the long run, glucocorticoid hypersecretion is considered a risk factor for a range of detrimental health outcomes in animals and in humans, such as the metabolic syndrome. In this study, a group of highly stressed persons born during World War II was compared to a "low" stress group of the same cohort on a range of psychological and medical variables.
Material/method(s): Subjects completed a set of questionnaires addressing pre- and postnatal influences, quality of parental bonding, adult stress reactivity, and medical conditions. It is hypothesized that depending on the time of stress occurrence during pregnancy, prenatally stressed adults will display an enhanced prevalence of diseases such as hypertension, visceral adiposity, and diabetes type II. Quality of parental bonding as a marker of the postnatal environment might mediate such relations. Furthermore, psychological responses to stress are compared to a non-stressed control group.
Result(s): Results will be presented at the conference.

Disclosure: Was this work supported by industry? No.

F-19
Impact of breastfeeding during the neonatal period on psychomotor and cognitive development in children of diabetic mothers

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Objective: In general, breastfeeding positively influences development of psychomotor function and cognition in children. Offspring of diabetic mothers (ODM) have a delayed psychomotor and cognitive development. Recently, we observed a negative impact of early neonatal ingestion of breast milk from diabetic mothers (diabetic breast milk, DBM) on overweight risk during early childhood. Here, we investigated the influence of neonatal DBM ingestion on neurodevelopment in ODM.
Materials/methods: A total of 242 ODM were evaluated for age of achieving major developmental milestones ('Denver Developmental Scale') according to early and late neonatal ingestion of DBM using Kruskal–Wallis test, Kaplan–Meier analysis, and Cox models.
Results: Early neonatal DBM ingestion positively influenced psychomotor development ($p=0.002$). Contrastingly, a delay in the onset of speaking was observed in children who had ingested larger volumes of DBM compared to those with lower DBM intake ($p=0.002$). Multivariate Cox models revealed a particular impact of DBM volume ingested early neonatally. However, a deleterious effect of breastfeeding after the first week on onset of speaking could not be excluded.

Conclusions: Ingesting larger compared to smaller volumes of DBM may normalize early psychomotor development in ODM, but delays onset of speaking which is an important indicator of cognitive development. This effect may result from qualitative alterations in DBM composition. Further studies are recommended urgently on benefits and harms of breastfeeding in ODM.
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Disclosure: Was this work supported by industry? No.

F-20
Endogenous pain inhibitory pathways are altered in offspring of mothers exposed to a bacterial mimetic in pregnancy

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Objectives: It has become clear that adverse early life experiences affect neurodevelopment and may cause permanent changes to behaviour. In animals, research suggests changes to behavioural pain responses following prenatal stress exposure. The aims of this study were to explore the effect of a bacterial endotoxin on later pain sensitivity and pain inhibition.
Methods: Pregnant *F344* rats were administered endotoxin (200 μg/kg, s.c.) or saline on gestational days 16, 18, and 20 (term = 23 days). The adult offspring experienced either a cold water swim or restraint to produce a stress-induced analgesia (SIA) response. SIA produced following a cold water swim is mediated by opioid inhibitory pathways, while restraint is mediated by opioid and non-opioid factors. Pain thresholds were assessed with the tail immersion task prior to and following stress exposure.
Results: Offspring of endotoxin-treated mothers showed no change in pain thresholds immediately following the cold water swim stress, while offspring of control animals showed a significant SIA ($p < 0.05$). Furthermore, this effect was exaggerated in the male offspring. Following the restraint stress, the offspring of endotoxin-treated mothers showed a significantly elevated SIA response, compared to control offspring, with decreased pain thresholds ($p < 0.05$).
Conclusion: These results suggest that maternal exposure to a bacterial mimetic during pregnancy affects pain inhibitory pathways differentially, resulting in long-term deficits to opioid inhibitory pain control. This study suggests that the prenatal environment is a critical mediator of long-term pain perception and may have implications for clinical pain management.

Disclosure: Was this work supported by industry? No.

F-21
Birth weight and head circumference are associated with stress-related movement behaviour in 8- to 9-year-old boys

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Objective: To assess whether anthropometric measures at birth across a normal range are associated with movement behavior during a stress test in children, and if stress-related movement behavior is associated with hyperactivity and attention in everyday life.
Methods: Healthy children, followed since 12 weeks of gestation, were asked to stand in front of a video camera and perform a story of their own invention followed by a serial subtraction task while standing still in front of an audience of three adult strangers. For 103 children (52 boys, 51 girls; 8—9 years), movement scores were recorded from video tapes by two observers. Hyperactivity during everyday life was measured by mother's reports on the Strengths and Difficulties Questionnaire (SDQ). Weight (BW), head circumference (BHC), and crown-heel length (BL) were measured at birth.
Results: Ordered regression analysis revealed an inverse association of HCB ($p = 0.017$) with movement rate during stress. Sex-specific analyses showed significant inverse effects in boys (BW: $p = 0.029$, HCB: $p = 0.010$, ponderal index: $p = 0.040$), but not in girls. Effects were stable controlling for gestational age and potentially confounding maternal factors. In boys, movement rate scores during stress showed a weak positive association with the SDQ hyperactivity scale ($r = 0.21$).
Conclusions: Size at birth is inversely associated with stress-related movement behavior in boys. Fetal nutrition and prenatal stress affect birth size and permanently alter dopaminergic function in neural motor networks during brain development, potentially influencing the emergence of hyperactivity problems in childhood.

Disclosure: Was this work supported by industry? No.

F-22
Birth parameters and cerebrovascular disease: a neuroimaging study

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Objective: Lower birth weight is associated with increased risk of stroke, but there is a need for more sensitive outcome variables. Vascular risk factors should be considered. We investigated the relationship between early life parameters and cerebrovascular disease (CVD) using neuroimaging and non-invasive measures of atheromatous load.
Methods: 110 community-dwelling subjects (70.0% female, mean age 78.2 (S.D. 1.4) years) born in Edinburgh hospitals between 1921 and 1926 had *birth parameters*—weight (BW), length (BL), placental weight (PW)—extracted from archives. *Atheromatous load* was measured by carotid intima media thickness (CIMT) and ankle brachial pressure index (ABPI). *Neuroimaging* included white matter lesions (WML) and diffusion tensor imaging (DTI) parameters: increased mean diffusivity $\langle D \rangle$ and decreased fractional anisotropy (FA) indicate less WM tract integrity, i.e. more disease.

Results: There was a trend towards a negative association between WML load and BW ($\rho = -0.17$, $p = 0.09$), and a significant negative association with PW ($\rho = -0.29$, $p = 0.008$). DTI parameters had a similar pattern (BW: frontal $\langle D \rangle$ $r = -0.08$ $p = 0.044$; FA $r = 0.20$, $p = 0.04$; PW: $\langle D \rangle$ $r = -0.25$, $p = 0.03$; FA $r = 0.36$, $p = 0.001$). There was no association between birth parameters and CIMT or ABPI ($r = -0.08$ to 0.05, $p > 0.4$).
Conclusion: In this sample lower placental weight, and possibly lower birth weight, were associated with sensitive measures of WM tract damage (increased WML load, increased $\langle D \rangle$, decreased FA). This was not directly due to atheromatous load, and the mechanism of conversion of atherosclerosis to atherothrombosis may be important.

Disclosure: Was this work supported by industry? No.

F-23
Birth parameters and cognitive ability in older age

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Objective: There is a small but statistically significant association between birth weight and cognitive ability in childhood and early adulthood, independent of social class. Few studies have examined the relationship into old age. We investigated the relationship between birth parameters and cognitive ability aged around 80 years.
Methods: 110 community-dwelling subjects (70.0% female, mean age 78.2 (S.D. 1.4) years) born in Edinburgh hospitals between 1921 and 1926 had birth parameters (weight, length, placental weight) extracted from archives, and underwent neuropsychological tests, including NART (estimate of prior ability), MMSE (test of global cognitive ability), and Raven's progressive matrices (test of non-verbal reasoning).
Results: There was a significant association between birth weight (corrected for gestational age) and cognitive ability in old age (Raven's $r = 0.25$, $p = 0.01$; MMSE $\rho = 0.21$, $p = 0.04$). There was no significant association between birth weight and estimated prior ability (NART $r = 0.12$, $p = 0.17$). The birth weight—Raven association, corrected for NART, was $r = 0.19$, $p = 0.06$. Parental social class correlated negatively with estimated prior cognitive ability (NART $\rho = -0.21$, $p = 0.02$) but not with cognitive ability in later life (Raven's $\rho = -0.09$, $p = 0.36$).
Conclusions: In this sample, there was a small but statistically significant association between birth weight and cognitive ability aged almost 80 years. The prenatal environment may influence cognitive ability into old age. This was not explained by prior cognitive ability or social class at birth. However, this sample was small and at risk of bias.

Disclosure: Was this work supported by industry? No.

F-24
Maternal care influences growth and metabolism at different ages

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Objectives: The quality of the precocious environment and the rate of growth are determinants of later adaptation to specific challenges, stress responses, and susceptibility to disease. We studied if naturally occurring variations in maternal care could affect growth and hormones such as GH, leptin, and insulin.
Materials and methods: Long—Evans rats were categorized as receiving low or high levels of maternal licking and grooming (low-LG and high-LG) during the first days of life. Hormones were measured at day 4 and at 3 months. The rate of growth was measured weekly from days 1 to 21.
Results: Low-LG rats displayed increased rate of growth from days 1 to 21, without reaching the body weight (BW) values from High-LG rats on day 21st. At 3 months, there were no more differences in BW. At day 4, low-LG rats showed increased plasma levels of insulin but no differences in leptin, GH, glycemia, or the HOMA index. Linear regression showed an inverse correlation between the LG score and insulin ($\beta = -0.269$, $p = 0.038$). At 3 months, there were no differences in insulin, GH, glycemia, or the HOMA index, although high-LG rats presented increased abdominal fat deposition to BW ratio (mean $\pm$ S.E., 1.93 ± 0.23 for low-LG and 2.99 ± 0.24 for high-LG, $p = 0.007$, Student's t-test) and increased leptin levels (4.25 ± 3.32 for low-LG and 8.87 ± 4.80 for high-LG, $p = 0.047$).
Conclusions: Early maternal care alters growth patterns and related hormones, which could be associated with specific advantages for adaptation to different environments and levels of stress in adulthood.

Disclosure: Was this work supported by industry? No.

F-25
Severe prematurity is not associated with increased ADHD symptoms in young adulthood

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Objectives: Severe prematurity is associated with increased prevalence of attention deficit hyperactivity disorder (ADHD) in childhood. We studied whether ADHD symptoms are more frequent in adults born severely preterm.
Methods: At age 18—27, 161 very low birth weight individuals (VLBW, <1500 g) and 171 term-born controls filled in the Adult Problem Questionnaire (De Quiros and Kinsbourne, 2001), which is a 43-item self-rating scale assessing adult ADHD symptoms. Each item is rated from 0 to 3, with higher scores indicating more symptoms. In the VLBW group, 110 were AGA (appropriate for gestational age: ≥ -2 birth weight S.D.) and 51 were SGA (small for gestational age: < -2 S.D.).

Results: Within term-born men, mean ADHD scores were 39.6 (S.D. 21.1). Men in the VLBW-AGA group had lower scores (mean difference with term −12.3, 95% CI −19.1 to −5.4, $p=0.001$), whereas no difference was seen with VLBW-SGA men (mean difference with term −3.0, −12.1 to 6.1, $p=0.5$). Within term-born females, mean ADHD scores were 45.6 (S.D. 18.6). No significant difference was seen with VLBW-AGA (−3.7, −9.3 to 1.9, $p=0.2$) or VLBW-SGA females (2.4, −4.9 to 9.6, $p=0.5$). Interaction p-values were 0.06 for sex*VLBW-AGA and 0.4 for sex*VLBW-SGA.
Conclusions: Young adult men born VLBW-AGA have lower ADHD symptom scores than term-born control men. No similar difference is seen in men born VLBW-SGA or within women. The result contrasts with the increased prevalence of childhood ADHD in preterm survivors, which may indicate that preterm children's ADHD differs from common ADHD and that this difference becomes apparent in adulthood.

Disclosure: Was this work supported by industry? No.

F-26
Sleep quality in young adults born severely preterm

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Objectives: Recent evidence suggests that severely preterm birth is associated with elevated blood pressure and impaired glucose regulation in young adult life. Because these conditions are associated with poor sleep quality, our aim was to assess sleeping patterns in young adults born severely preterm.
Methods: The cohort consists of 166 very low birth weight subjects (VLBW, <1500 g) and 173 term-born controls. Sleep quality was assessed in 215 individuals at age 19—27 years, using actigraphs which register motor activity. Using the registered data, sleep quality (i.e. sleep length and latency) can be reliably assessed. Adequate sleep data was gathered for 180 individuals (89 controls and 91VLBW-subjects). Individuals with cerebral palsy were excluded ($n=7$).
Results: Within the control and VLBW groups respectively, the average (± S.D.) sleep length (7.19 h ± 0.88 h vs. 7.21 h ± 1.05 h, $p=0.60$) and sleep latency (0.24 h ± 0.23 h vs. 0.32 h ± 0.39, $p=0.37$) were similar. Gestational age correlated with sleep latency among controls ($r=-0.30$, $p<0.01$) but not among VLBW-subjects ($r=0.02$, $p=0.85$). Sleep length did not correlate between gestational age in either group. When controlling for age, sex, and parental education, the effect of gestational age on sleep latency remained significant only among controls (control: $B=-0.05$, $p=0.01$; VLBW: $B=-0.02$, $p=0.29$). Intrauterine growth retardation did not affect the sleep latency or sleep duration within the VLBW-group.
Conclusions: Prematurity was not related to sleep quality in young adulthood. However, in term-born subjects, gestational age was associated with later sleep onset problems.

Disclosure: Was this work supported by industry? No.

F-27
Prenatal maternal anxiety predicts flattening of the day-time cortisol profile and depression in adolescence

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Objective: Experimental animal research testing the perinatal programming hypothesis has demonstrated that dysregulation of HPA axis may result from perinatal stress and can lead to depressive-like behavior later in life. The aim of the present study is to investigate the link between maternal anxiety during pregnancy, HPA axis functioning, and depression in the mid-adolescence in humans.
Materials/methods: Our prospective-longitudinal study started with 86 healthy mothers and their firstborn children. The sample has been assessed at 12—22, 23—31, and 32—40 weeks postmenstrual age and at 1, 10, 28 weeks and at ages 8—9, 14—15, and 17 after birth. Besides other variables, maternal anxiety was measured on all occasions, using the State Trait Anxiety Inventory. HPA axis functioning was measured through establishing a saliva day-time cortisol profile (shortened version); severity of depressive symptoms was measured with the Children's Depression Inventory (CDI).
Results: Regression analyses indicated that maternal anxiety at 12—22 weeks postmenstrual age was in both sexes associated with a day-time cortisol profile showing reduced variability, and in female adolescents also with depression. The flattened profile was mainly due to elevated evening cortisol. Effects remained when controlling for postnatal maternal anxiety; smoking, birth weight, obstetrical optimality, maternal postnatal anxiety, and puberty phase had no effect.
Conclusion: Fetal programming of the HPA axis, induced by prenatal maternal anxiety, is a mechanism that may underlie enhanced susceptibility to depression.

Disclosure: Was this work supported by industry? No.

F-28
Prenatal stress exposure predicts insulin resistance in adult life

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Objectives: Epidemiological studies in humans have uncovered robust associations between birth phenotype and subsequent risk for insulin resistance (IR) and type 2 diabetes mellitus (T2DM). It is unlikely that birth phenotype plays a *causal* role; it more likely represents a marker of intrauterine conditions that also program physiological processes in the developing fetus. Exposure to high levels

of prenatal stress has been proposed as one such intrauterine condition.
Methods: 36 healthy young adults whose mothers experienced severe stress during pregnancy (e.g. death in the family, loss of home; prenatal stress (PS) group) and comparison group ($n=22$, CG group) underwent an oral glucose tolerance test after fasting overnight.
Results: Glucose levels did not differ significantly between the two groups, however PS subjects had significantly higher basal insulin levels, higher 2 h insulin and C-peptide levels, and a trend for higher HOMA-IR insulin resistance. This association of PS with insulin and C-peptide levels remained significant after controlling for BMI, and was independent of birth phenotype, family history of T2DM, lipid profile, pro-inflammatory state and behavioral risk factors.
Conclusion: Higher insulin secretion to a glucose load is a reflection of relative insulin resistance in these young adults who were exposed to stress *in utero* and may predispose them to develop T2DM later in life. To the best of our knowledge, this study is the first to demonstrate a direct link in humans between prenatal stress exposure and alterations in glucose-insulin-related metabolic function in adult life.

Disclosure: Was this work supported by industry? No.

F-29
Cortisol administration to pregnant sows affects behaviour of their piglets

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Objective: Repeated oral administration of hydrocortisone-acetate (HCA) to 41 pregnant sows was used as a model for prenatal stress.
Methods: HCA was orally administered to pregnant sows during one of three periods of gestation: 21—50 (P1, $n=10$), 51—80 (P2, $n=10$), and 81—110 (P3, $n=10$) days after insemination (term 115 days). Control sows (C, $n=11$) received vehicle from 21 to 110 days after insemination.
Results: HCA piglets weighed less at birth and at weaning, compared to control piglets. Between days 9 and 48 after birth, treatment effects on male and female piglet behaviour were determined by observations in the home pen and by means of five different behavioural tests. During a backtest and a tonic immobility test, no gender differences were observed in vocalisations in HCA piglets, while control males vocalised more than control females. In the home pen at 2 weeks of age, HCA piglets spent less time in social interactions than control piglets. During the novel environment test, P1 and P3 piglets walked more than control piglets. During a mixing test, P1, P2, and P3 piglets had fewer non-aggressive encounters, and P2 piglets continued fighting longer than control piglets.
Conclusions: Elevated maternal cortisol concentrations during gestation affect piglet behaviour, and some of these effects do differ between male and female piglets. In addition, effects depend on the period of cortisol administration to the mother.

Disclosure: Was this work supported by industry? No.

G. GLUCOCORTICOIDS, MEDICATION, AND TOXINS

G-01
Differential effects of maternal betamethasone on ovine placental lactogen and binucleate cell number in sheep placentome subtypes

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Objective: Reduced fetal weight after maternal betamethasone (BET) exposure may be mediated through placental effects. Placental binucleate cells (BNC) produce ovine placental lactogen (oPL), a placental growth factor. We investigated the effects of maternal BET injections on oPL protein levels and BNC numbers and distribution in ovine placentome subtypes.
Methods: Pregnant ewes carrying male fetuses were injected with saline or 1 (104 days of gestation; dG), 2 (104, 111dG), or 3 (104, 111, 118dG) doses of betamethasone (beta) (0.5 mg/kg). Placental tissue was dissected and classified into A-, B-, C-, and D-subtypes prior to (75, 84, 101 dG), during (109, 116 dG), and after BET at 121—122, 132—133, and 146—147 dG. oPL localization (Immunohistochemistry) and protein levels (Western blot) were determined and BNCs counted.
Results: BNC numbers in control placentae were lowest at 146 dG compared to other gestational ages. In A-types, BNC numbers increased from 75 to 146 dG and oPL protein reached maximum levels at 109 dG. In B- and C-types, BNC number increased from early (75 dG) to mid- (121 dG) gestation and then decreased at 146 dG. In BET placentae, oPL BNC numbers were decreased by ~ 50% in A-, B-, and C-types across gestational ages compared to controls; oPL

protein levels increased from 132 dG to term in BET groups.
Conclusion: Our data demonstrate that BNC number is differentially regulated in ovine placentome subtypes and differ with gestational age. Maternal BET injections persistently reduced BNC numbers in all placentome subtypes. Maternal BET acutely suppresses oPL protein levels mid-gestation followed by a functional recovery at term.

Disclosure: Was this work supported by industry? No.

G-02
Influences of early maternal dexamethasone on fetal growth in sheep

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Objective: Fetal exposure to bioactive glucocorticoids (GC) occurs at an appropriate time in gestation for maturation. We hypothesised that exposure to GC early in pregnancy would expose the fetus to GC at developmentally sensitive windows and would result in developmental programming.
Methods: Pregnant ewes with singleton pregnancies (total $n=119$) were randomised to control or dexamethasone (Dex, 4 intramuscular injections of 0.14 mg/kg 12 h apart) groups and were injected with saline or Dex from 40 days of gestation (dG). Fetal weights and anthropometric measures were recorded at 50, 102, 126, 141 dG, and correlated to fetal weight.
Results: Control and treated males were bigger than females (102*, 141* dG). Control females were longer (crown rump length) and had larger abdominal circumference than males at 102* and 141* dG. Dex exposed fetuses had reduced weights at 102*, 126 and 141 dG and increased total brain weight at 102* dG compared to control. Total adrenal weight in females was reduced in Dex fetuses at 126* and 141 dG, compared to controls. Control females had larger adrenals than males at 126* and 141* dG, but this sex-specific effect was not observed in Dex-treated animals. Hippocampal, pituitary, cerebellum, heart, liver, pancreas, kidney, and perirenal fat weights were similar between groups. * indicates $p<0.05$.
Conclusions: Early fetal Dex exposure resulted in fetal and organ weight changes that persisted to term. It appears that this time in gestation represents a developmentally sensitive window resulting in the programming of growth for the remainder of gestation.

Disclosure: Was this work supported by industry? No.

G-03
Comparative effects of antenatal betamethasone and dexamethasone on endocrine and reproductive function in adult female offspring

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Introduction: Approximately 7% of pregnant women are treated with synthetic glucocorticoids (sGC) to promote lung maturation in fetuses at risk of premature delivery. We hypothesized that fetal exposure to sGC would adversely affect endocrine and reproductive function in adult female offspring and that these effects differ for different formulations of sGC.
Methods: Pregnant guinea pigs were treated with betamethasone (BETA, 1 mg/kg), dexamethasone (DEX, 1 mg/kg), or vehicle (VEH) on gestational days (gd) 40/41, 50/51, 60/61 (term ~ 70 days, $n=11-13$/gp). Female offspring were monitored until adulthood, when hypothalamo–pituitary–adrenal (HPA) activity was assessed at different stages of the reproductive cycle (~16 days). Females were then mated with control males, and HPA activity assessed through pregnancy. After weaning, mothers were euthanized in the luteal phase.
Results: BETA females exhibited significantly lower salivary cortisol during the luteal phase. In contrast, cortisol was higher ($p<0.05$) in BETA females during a moderate stress, but only in estrous. HPA function was not significantly altered in DEX females. BETA but not DEX exposed females took significantly longer to become pregnant ($p<0.05$). In pregnancy, cortisol rose towards term ($p<0.01$) in VEH and DEX females but not in the BETA group. Plasma estradiol levels were significantly lower in BETA but not DEX females during the luteal phase.
Conclusion: sGC have profound influences on endocrine and reproductive function. Further, our results indicate that the long-term effects of BETA are far more extensive than those for DEX. This is important as BETA is becoming the sGC of choice for promoting fetal lung maturation.

Disclosure: Was this work supported by industry? No.

G-04
Pre- and postnatal tobacco smoke exposure and age at menarche

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Objectives: Despite the abundance of research on environmental exposures in early childhood and age at menarche, limited research has been done on the effects of intrauterine exposures on age at menarche. Data were analyzed from the New York site of the National Collaborative Perinatal Project (NCPP) to assess the effects of prenatal and postnatal tobacco smoke exposure on age at menarche.
Materials and methods: We contacted former female participants of the New York site of the NCPP who were born between 1959 and 1966 and prospectively followed for

7 years. To date, we have successfully contacted and obtained epidemiologic data, including age at menarche, on 262 women. Logistic regression was used to compare later menarche (≥13 years) to earlier menarche (<13 years).
Results: Adjusting for maternal age at menarche, maternal education, maternal age at pregnancy, pre-pregnancy weight, infant race, birth weight, weight at age 7 and height at age 7, the odds ratio for the association between the highest category of prenatal tobacco smoke exposure (≥20 cigarettes/day) and later age at menarche was 1.9 (95% CI: 0.8, 4.7). Exposure to both prenatal and postnatal tobacco smoke exposure was associated with later age at menarche (OR=2.3, 95% CI: 1.1, 4.6).
Conclusions: These results suggest that prenatal and postnatal tobacco smoke exposure may delay age at menarche.

Disclosure: Was this work supported by industry? No.

G-05
Developmental changes in ovine adrenal gene expression after exposure to prenatal betamethasone (BETA)

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Objective: To determine how alterations in adrenal gene expression contribute to hypothalamic—pituitary—adrenal (HPA) programming, we investigated effects of maternal beta on pre- and postnatal ovine adrenal gene expression.
Methods: Pregnant ewes carrying male fetuses were injected with 1 (104 days of gestation, dG), 2 (104, 111 dG), or 3 (104, 111, 118 dG) doses of beta (0.5 mg/kg) or saline. Adrenal tissue was collected at 84, 109, 116, 132, and 146 dG and at 6 and 12 weeks of age. Levels of mRNA of P450c17 and 3βHSD, ACTH (ACTHr) and glucocorticoid (GR) receptors, StAR, and 11βHSD2 were determined using qRT-PCR.
Results: In controls, levels of StAR, ACTHr, P450c17, and 11βHSD2 mRNA increased with gestation. Exposure to beta decreased levels of StAR at 132 and 146 dG, but increased ACTHr levels at 146 dG. P450c17 mRNA levels were decreased at 109 and 116 dG but elevated at 6 weeks. 3βHSD levels were increased at 132 and 146 dG. Beta decreased 11βHSD2 mRNA levels at 12 weeks. GR mRNA levels were similar across all ages and unaffected by beta. Repeated maternal beta increased gestation length by 2 days compared to controls (151.7 ± 0.5, 153.6 ± 0.6).
Conclusions: Levels of genes controlling fetal adrenal steroidogenesis increased with gestation, likely facilitating a rise in HPA activity at term. Reduced 11βHSD2 levels at 12 weeks signify persisting effects of prenatal beta after birth. Interpretation of these data is complicated by delayed labour in beta-treated animals. Together, these findings most likely indicate that prenatal beta treatment has altered the trajectory of peripartum HPA activity.

Disclosure: Was this work supported by industry? No.

G-06
Repeated maternal glucocorticoid treatment affects activity and hippocampal NMDA receptor expression in juvenile guinea pigs

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Objectives: Behavioural consequences of prenatal synthetic glucocorticoid exposure are not well-understood, though emerging studies in humans indicate hyperactivity and altered cognitive development. Recent reports indicate that *N*-methyl-D-aspartate receptors (NMDAR) may mediate the development of postnatal stress behaviours. We hypothesized that prenatal betamethasone (BETA) administration would alter behaviour and the expression of hippocampal NMDAR subunits NR1, NR2A, and NR2B in juvenile guinea pig offspring.
Methods: Pregnant guinea pigs were treated with BETA (1 mg/kg, $n=13$) or vehicle ($n=15$) on gestational days 40/41, 50/51, 60/61 (term 70 days) and animals delivered normally. At 10 days of age, offspring activity in an open-field was assessed by computerized tracking. NMDAR subunit expression was assessed by in situ hybridization and Western analysis.
Results: BETA had no significant effect on birth weight or early growth. However, it produced sex-specific effects in open-field activity and hippocampal NMDAR expression compared to controls. Female BETA offspring exhibited significantly increased locomotor activity while there was no effect in BETA males. BETA males exhibited a tendency for decreased anxiety-like behaviour. BETA-exposed females exhibited significantly reduced NR1 mRNA in CA1/2 and CA3 sub-fields of the hippocampus; there were no effects in males.
Conclusion: Repeated maternal treatment with BETA in a similar regimen to that administered to pregnant women at risk of delivering preterm has profound consequences on behaviour and development of crucial neurotransmitter systems in postnatal life.

Disclosure: Was this work supported by industry? No.

G-07
Effects of antidepressants during pregnancy on the fetus

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Objective: Many pregnant women are treated with antidepressant drugs, while little is known about the safety for the child. Nowadays, the most frequently prescribed antidepressant drugs during pregnancy are the selective serotonin

reuptake inhibitors (SSRIs). It is unknown whether this medication affects the development of the central nervous system of these children and what the long-term consequences are. Therefore, the effects of different SSRIs during pregnancy on mouse development and their long-term consequences are addressed in this study.
Materials/methods: Pregnant C57Bl6/Jico mice were injected daily (i.p.) either fluoxetine, fluvoxamine, or saline to determine the long-term effects of a SSRI with relatively high placental transfer (fluoxetine) and low transfer (fluvoxamine). Physiological and behavioral analysis was performed on the offspring at adult stage.
Results: Fluoxetine treatment decreased the survival rate of the offspring by 81%. Moreover, fluoxetine-treated mice are more anxious at adult stage, while fluvoxamine-treated mice did not show differences in behavior at adult stage.
Conclusions: Prenatal fluoxetine treatment affects the survival rate of the offspring. These preliminary results also show that fluoxetine-treated mice are more vulnerable to anxiety disorders at adulthood. Fluvoxamine has no long-term effects on behavior and may be a safer antidepressant during pregnancy compared to fluoxetine.

Disclosure: Was this work supported by industry? No.

G-08
Long-term effects of adverse environments during development: effects in adulthood in rats exposed to toxicants or undernutrition in utero

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Studies have shown correlations between in utero and early life environments and diseases later in life, including hypertension, coronary heart disease, diabetes, obesity, schizophrenia, early onset chronic renal failure, cancer, and compromised reproduction. Current developmental toxicology studies do not assess long-term health in offspring. We sought to determine if toxicant exposure of pregnant rodents would have long-term health effects on offspring.
Methods: We exposed pregnant rats to perfluorooctane sulfonate, atrazine, or dexamethasone in regimens designed to result in reduced birth weight. Another group was subjected to undernutrition. After birth, each group was subdivided into large litter ($n=12$) or small ($n=6$), and health outcomes of offspring was monitored in adulthood.
Results: Maternal exposure to drug, chemicals or undernutrition resulted in lower birth weight; recovery of body weight was noted, with those in small litters recovering sooner. Male offspring had higher blood pressure than controls as early as 7 weeks of age, while female offspring did not exhibit elevated blood pressure until later, 37 weeks of age. Rats reared in small litters had slightly higher blood pressure than those reared in large. We have also measured serum glucose and insulin responses after glucose challenge of offspring, and results will be reported.
Conclusions: These studies indicate that elevated blood pressure may be a common sequela of prenatal toxicant exposure. Ongoing studies are designed to elucidate the potential for early chemical exposure to affect adult health and disease susceptibility.

Disclosure: Was this work supported by industry? No.

G-09
Developmental changes in hippocampal corticosteroid receptors and 11βhydroxysteroid dehydrogenase in fetal sheep: effects of glucocorticoid exposure

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Objective: Glucocorticoids (GC) program fetal hypothalamo–pituitary–adrenal (HPA) activity. We determined the effects of prenatal GC on fetal and postnatal ovine hippocampal corticosteroid receptors (GR, MR) and 11βHSD mRNA levels.
Methods: Pregnant ewes carrying male fetuses were injected with saline or 1 (104 days of gestation, dG), 2 (104, 111 dG), or 3 (104, 111, 118 dG) doses of betamethasone (beta) (0.5 mg/kg). Hippocampal tissue was collected prior to (75, 84, 101), during (109, 116), and after beta at 121, 132, and 146 dG, and at 6 and 12 weeks of age. Levels of GR, MR, and 11βHSD1 mRNA were determined using quantitative RT-PCR.
Results: In control animals, GR mRNA levels increased from 75 dG to max values at 109 dG* and decreased thereafter to 6 weeks (*$p<0.05$). Levels increased again at 12 weeks*. 11βHSD1 mRNA showed a similar pattern; increasing from 75 dG to 109 dG* then decreasing to 146 dG* and remained low in postnatal life. MR mRNA remained constant throughout the ages studied; with the exception of 116 dG*, when levels were low. Beta exposure resulted in acute effects on GR and MR mRNA, increased levels at 116 dG*. Beta exposure increased 11βHSD1 levels in offspring at 12 weeks*, without fetal effects.
Conclusions: During rapid fetal brain growth, GR, and 11βHSD1 levels are high, facilitating neuronal exposure to GC induced maturation. Levels decrease thereafter, allowing increased HPA activity late in gestation. Fetal hippocampal GR mRNA does not appear to be auto-regulated. However, beta exposure may alter GR and MR developmental trajectories and increase postnatal neuronal exposure to bioactive GC through increased 11βHSD1, possibly altering postnatal HPA activity.

Disclosure: Was this work supported by industry? No.

G-10
Hippocampal gene expression is altered in adult sheep offspring after prenatal betamethasone exposure

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Objective: Prenatal betamethasone (beta) exposure reduces brain weight in adult sheep offspring and suppresses long-term hypothalamic—pituitary—adrenal (HPA) function. Since HPA activity is regulated by hippocampal corticosteroid receptors, we determined effects of prenatal beta on hippocampal receptor and 11βhydroxysteroid dehydrogenase (11βHSD) expression in adult sheep offspring.
Methods: Pregnant ewes or their fetuses received either repeated intramuscular saline (MS, FS) or beta injections (0.5 mg/kg; M4, F4) at 104, 111, 118, and 124 days of gestation (dG), or a single beta injection at 104, followed by saline at 111, 118, and 124 dG (M1, F1). Hippocampal tissue was collected from offspring (3.5 years). Levels of glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) and 11βHSD1 and 2 mRNA were determined using qRT-PCR.
Results: Single and repeated maternal beta injections increased MR mRNA levels ($p<0.001$) and repeated injections increased 11βHSD2 mRNA levels ($p<0.040$) in adult offspring at 3.5 years of age. Single but not repeated fetal beta injections decreased MR mRNA levels ($p<0.05$) and single and repeated injections decreased 11βHSD1 mRNA levels ($p<0.010$).
Conclusions: Increased MR mRNA levels in beta exposed offspring may underlie suppression of basal HPA function previously observed in these animals. Increased 11βHSD2 mRNA levels may lead to protection; reducing neuronal exposure to glucocorticoids. Decreased 11βHSD1mRNA levels may have the same neuro-protective effects; decreasing cellular exposure of bioactive glucocorticoids.

Disclosure: Was this work supported by industry? No.

G-11
Reduced life expectancy in rats after neonatal dexamethasone treatment

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The glucocorticosteroid dexamethasone is widely used in preterm infants for the prevention of chronic lung disease. However, major concern has arisen about the long-term sequelae of this therapy.
Here we report that neonatal treatment with dexamethasone significantly shortens life span in rats, notably with 25% in males and 18% in females. Histopathological examination indicated end stage cardiac and renal failure as the cause of premature death. Already at young adult age, dexamethasone-treated rats showed symptoms of hypertension that increased with age.
Thus, a brief period of glucocorticosteroid treatment during early life results in untimely death presumably due to cardiovascular and renal disease later in life. These serious, adverse long-term consequences call for utmost prudence with glucocorticosteroid treatment of human preterm infants and careful follow-up of young adults with a history of neonatal glucocorticosteroid treatment in early life.

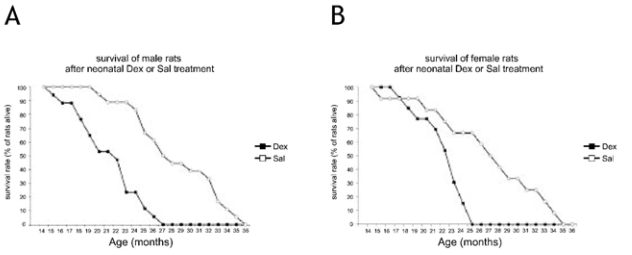

Figure 1 A: Survival rate of male rats neonatally treated with dexamethasone (Dex, n=17) or saline (Sal, n=18). Dex rats show reduced life span compared to Sal rats (p<0.0001, Kapla-Meier survival analysis). B: Survival rate of female rats neonatally treated with dexamethasone (Dex, n=13) or saline (Sal, n=12). Dex rats show reduced life span compared to Sal rats (p<0.005, Kaplan-Meier survival analysis).

Disclosure: Was this work supported by industry? No.

G-12
Renal effects of acute cortisol infusion in ewes hypertensive as result of early glucocorticoid treatment

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Objectives: Pregnant ewes, treated with cortisol or dexamethasone for 48 h early in pregnancy (26—28 days, term 150 days) give birth to lambs which develop high blood pressure after 4 months, and which have reduced nephron endowment from birth. The aims of the current experiments were to assess basal renal function in adult female offspring, and the response to infused cortisol (5 mg/h for 6 h), which is known to increase renal plasma flow in normotensive sheep, by alterations in nitric oxide/prostaglandin systems.
Methods: Conscious control, saline pretreated (C, n = 5), dexamethasone pretreated (D, n = 8), and cortisol pretreated (F, n = 8) ewes had measurements of glomerular filtration rate (GFR), renal plasma flow (RPF), and electrolyte excretion rates during 6 h infusions of neither saline (12 ml/h) or cortisol (5 mg/h), after a 1 h control period. All animals were 5—6 years old, and body weights were similar (C, 52 ± 3; D, 52 ± 2; F, 54 ± 2 kg).
Results: Basal GFR was maintained in all groups, despite a 30—40% decrease in nephron number in groups D and F. Thus, single nephron filtration rate must have been increased in the latter groups. Cortisol infusion caused a significantly bigger

increase (364 ± 55 ml/min) in RPF in F group, than in C (202 ± 62) or in D (189 ± 108), although the plasma cortisol concentrations were raised to equivalent levels (320—340 nM) in all groups.
Conclusions: Adult female sheep, exposed to high levels of cortisol for a brief period , in early development, have an altered renal response to short-term elevation of plasma cortisol, consistent with their being a greater capacity for nitric oxide/prostaglandin production in these kidneys.

Disclosure: Was this work supported by industry? No.

G-13
ACTH and cortisol responses of fetal sheep to umbilical cord occlusion in the presence of chronic intrauterine inflammation

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Objective: To examine the effects of chronic intrauterine inflammation on fetal hypothalamic—pituitary—adrenal (HPA) responses to umbilical cord occlusion (UCO).
Methods: Instrumented fetal sheep underwent a series of five UCOs (2-min duration, 30-min intervals) on days 117 and 118 of gestation (term is 150 days), after a 4-week intra-amniotic infusion (osmotic pump) of lipopolysaccharide (LPS, *E. coli* 055:B5, 1.1 mg/day, $n=6$) or saline (control, $n=6$) from 80 days. Adrenocorticotrophin (ACTH) and cortisol concentrations were measured, in serial plasma samples, by radioimmunoassay. Data were compared by *t*-test or repeated measures ANOVA and are presented as mean ± S.E.M.
Results: Amniotic fluid LPS infusion resulted in intrauterine inflammation that persisted until 125—126 days. Prior to UCOs (at 117 days), fetal ACTH concentrations were higher in the LPS group (28.8 ± 4.9 pg/ml) than control (15.1 ± 3.1 pg/ml: $p=0.02$); cortisol concentrations tended to be higher (LPS, 5.0 ± 0.8 ng/ml; control, 3.4 ± 0.3 ng/ml: $p=0.09$). ACTH responses to UCOs were not different between groups at 117 days, but at 118 days the elevations in ACTH concentrations in response to UCO were greater in the LPS group than control ($p<0.05$). Fetal cortisol responses to UCO were not significantly different between groups. Physiological responses to UCO were equivalent between groups.
Conclusion: Exposure to a pro-inflammatory intrauterine environment appears to alter basal fetus HPA axis function and influence the HPA response to UCO. These initial observations suggest that fetal adrenal sensitivity to increases in ACTH is attenuated by intrauterine inflammation.

Disclosure: Was this work supported by industry? No.

G-14
Fetal protection from excess glucocorticoid exposure: downregulation of placental 11beta-hydroxysteroid dehydrogenase

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Objective: Undernutrition or overexposure to glucocorticoid in utero induces growth retardation, resulting in offspring obesity. Placental 11β-hydroxysteroid dehydrogenase type1 (11β-HSD1) converts inactive cortisone into active corticosterone, thus potentiating transfer of maternal steroids to the fetus; 11β-HSD2 does the opposite and acts as the physiological feto-placental "barrier" to endogenous glucocorticoids. We determined the impact of MFR on maternal plasma corticosterone and placental expression of 11β-HSD enzymes.
Materials/methods: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy days 10 to 16. At e16, maternal plasma corticosterone levels and placental mRNA expression of 11β-HSD1 and 11β-HSD2 were determined. mRNA data is normalized to β-actin and expressed as deltaCT (higher amounts correspond to lower CT).
Results: MFR dams had significantly decreased body weight (244 ± 8 g vs. 277 ± 8 g, $p<0.05$) with increased plasma corticosterone levels (623 ± 84 vs. 332 ± 43 ng/ml, $p<0.05$) as compared to control dams. Placental 11β-HSD1 expression was significantly lower in MFR pregnancies (12.0 ± 0.3 vs. 9.0 ± 0.2 CT, $p<0.001$) as compared to controls. However, 11β-HSD2 expression was unaltered (8.4 ± 0.2 vs. 8.6 ± 0.3 CT).
Conclusions: MFR produces stress response in the mother as evidenced by elevated plasma corticosterone levels. Decreased expression of placental 11-HSD1 implies a relatively reduced capacity for conversion of inactive 11-dehydrocorticosterone to the active corticosterone. This alteration may serve as an important protective barrier, limiting transplacental passage of excess steroids to the fetus during periods of maternal stress.

Disclosure: Was this work supported by industry? No.

H. MATERNAL AND INFANT NUTRITION

H-01
Prenatal exposure to undernutrition and glucose and lipid metabolism in young adults in Rural Matlab, Bangladesh

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Objective: To examine the association between prenatal exposure to undernutrition during Bangladesh famine in 1974—1975 and abnormalities of glucose and lipid metabolism in early adulthood (27—31 years) in a rural area in Bangladesh.
Subjects and methods: Exposed ($n=68$) and non-exposed, born before ($n=81$) and after ($n=70$) the famine with comparable sex distributions were randomly selected from ICDDR,B Health and Demographic Surveillance area in Matlab, Bangladesh. Exposed subjects were born during or after the famine but were exposed to famine for at least 3 months or longer during foetal life. The non-exposed were born either immediately before or after the famine and had no prenatal exposure to famine. Plasma glucose concentrations and serum lipid concentrations were measured after overnight fast. Glucose concentration was again measured 2 h after 75 g oral glucose challenge.

Results: Fasting plasma glucose concentration and that of 120 min did differ significantly between the exposed and the non-exposed. However, the prevalence of impaired glucose tolerance (IGT) was three times higher in exposed than both non-exposed groups (IGT: 11.8% vs. 3.7/4.3%, $p<0.03$). Neither of the parameters of lipid profile nor the prevalence of lipid abnormality differed significantly between the exposed and the non-exposed.
Conclusion: These findings from the pilot study suggest that prenatal exposure to undernutrition is associated with an elevated risk of IGT in young adults in rural Bangladesh. These findings need to be confirmed in larger study.

Disclosure: Was this work supported by industry? No.

H-02
RHOA signal transduction: effects of maternal copper deficiency on progeny

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Objective: Maternal copper deficiency during pregnancy and lactation impairs development of the fetal cardiovascular system, inducing reduction in CCO activity in neonatal cardiac mitochondria. Effects of maternal copper deficiency on RhoA signaling in the vasculature are unknown. The objective of this study was to determine the effect of maternal copper deficiency on RhoA-dependent vascular smooth muscle signaling.
Materials/methods: Copper deficiency (CuD) was induced by maintaining dams on a diet containing 1 mg copper/kg diet from 3 weeks prior to pregnancy through the end of the lactation period. After weaning, CuD litters were maintained on the same diet. Control copper adequate (CuA) dams and litters were fed a diet containing 8 mg copper/kg diet during the same time periods. Mesenteric arteries were collected from CuD and CuA male offspring at 3 and 9 weeks of age. Expression of signaling proteins in the RhoA-dependent pathway was determined by Western blot.
Results: CCO activity in cardiac mitochondria was significantly lower in the 3-week-old CuD offspring. Protein expression of RhoA was significantly increased in CuD offspring at 9 weeks, with no significant difference at 3 weeks. No significant differences were found between offspring groups in the expression of Rho-kinase α, Rho-kinase β, MYPT-1, pMYPT-1, or CPI-17 at 3 or 9 weeks of age.
Conclusions: Reduced CCO activity in weanling cardiac mitochondria is evidence of impaired energy metabolism resulting from maternal copper deficiency. Maternal copper deficiency does not induce alterations in RhoA-mediated vascular signal transduction in copper deficient offspring.

Disclosure: Was this work supported by industry? No.

H-03
Does DAM size and feeding during pregnancy affect fetal organ weight?

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Large mass dams (La) give birth to heavier offspring than do low mass dams (Lo) of the same species. Dams well-fed (Wf) during pregnancy often produce heavier offspring than poorly nourished (Pf) dams. However, in meat production animal farming, dams consume some 70% of total feed and winter feed is expensive (when many production animal females are pregnant). There is interest by farmers seeking to improve farm profit to use lighter mass dams and to minimize feed intake during pregnancy. This trial seeks to determine the differences in offspring and grand-offspring performance from ewes of either La or Lo either Wf or Pf during pregnancy. 400 La and 400 Lo ewes were chosen from a population of 3000 Romney ewes. They were synchronized and artificially inseminated with semen from Suffolk rams. From day 21 of pregnancy to term, 1/2 of each of the lines were either Wf or Pf. Ewes were pregnancy scanned at about day 45 of gestation. At days 65, 100, and 140, five twin-bearing ewes were euthanased from each of the four size by feeding groups. Fetal weights and dimensions and fetal organ weights were recorded.
Lo/Pf dams produced lighter fetuses with lighter spleen and adrenals than the other three groups (5.8 vs. 6.3 kg, 6.3 vs. 8.0 g, 0.63 vs. 0.72 g). Lo/Pf and La/Wf fetuses had smaller liver and thymus. Pf fetuses had smaller thyroids.
The changes in fetal organ mass support investigation into whether lifetime physiology/productivity is modified by dam treatments.

Disclosure: Was this work supported by industry? Yes: Meat and Wool New Zealand.
Do you act as a consultant, employee or shareholder with this industry? No.

H-04
Adiponectin and leptin mRNA expression are upregulated in juvenile offspring following maternal nutrient restriction in late gestation

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Circulating levels of the adipokines, leptin, and adiponectin increase and decrease respectively with increasing adiposity in adults. Whilst leptin suppresses energy intake, adiponectin reduces insulin resistance. Maternal nutrient restriction (NR) impairs glucose-insulin homeostasis and enhances fat deposition in juvenile offspring.
Singleton bearing sheep received either 100% metabolizable energy (ME) (C, $n=7$) throughout pregnancy or 50% ME from day 110-term (NRL, $n=4$). Adiponectin and leptin mRNA abundances were quantified by qPCR using ovine-specific primers using 18S rRNA as an internal control. PAT was sampled from young adults at 1 year of age. All animal procedures were performed in accordance with U.K. legislation.
There was a nine-fold increases in leptin (C 1.0 ± 0.5, NRL 9.0 ± 3.7 a.u., $p<0.05$) mRNA abundance accompanying the greater adiposity of NRL offspring (C 7.7 ± 1.3, NRL 17.5 ± 4.3 g/kg body weight, $p<0.05$). Not only was

adiponectin expression positively associated with glucose area under the curve (R^2 0.57, $p<0.01$) in NR late offspring but adiponectin mRNA abundance increased eight-fold (C 1.0 ± 0.5, NRL 8.2 ± 1.5 a.u., $p<0.01$).
Maternal NR during late gestation promotes adipose tissue deposition leads to insulin resistance in the offspring which exhibit raised adipokine mRNA abundance. The latter may reflects an adiponectin resistant state or an early compensatory response.

Disclosure: Was this work supported by industry? No.

H-05
Differential effects of decreased and increased maternal food intake in late gestation on maternal metabolism and birth weight

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Human and animal studies have shown that maternal food intake in pregnancy critically affects fetal development. This study compared the effects of a 50% reduction or increase in maternal food intake over the final month of gestation on maternal metabolic homeostasis and birth weight, thus reflecting the variation in voluntary energy intake reported in recent studies of contemporary U.K. populations during pregnancy.
At 110 days gestation, twin bearing pregnant sheep were randomly allocated ($n=10$ per group) to control (C), or a 50% increase (I) or decrease (D) in food consumption. At ~130 days gestation, maternal blood samples were taken for measurement of plasma glucose and non-esterified fatty acids (NEFA). All mothers gave birth normally at term.
Although birth weight and in maternal plasma glucose were reduced by decreased maternal food intake (C 4.84 ± 0.15, D 3.81 ± 0.13, I 4.76 ± 0.17 kg: C 5.33 ± 0.50, D 3.47 ± 0.39, I 5.77 ± 0.50 mM, respectively), both were unaffected by increased food consumption. The latter, did, however, promote maternal weight gain to term (C 1.22 ± 0.03, D 1.08 ± 0.01, I 1.30 ± 0.03% mat. wt. at 110 days), in conjunction with lower plasma NEFA (C 0.39 ± 0.06, D 0.85 ± 0.06, I 0.20 ± 0.07 mM).
Increasing maternal food intake has no effect on birth weight but prevents the mobilisation of maternal body reserves in late gestation. The extent to which this may impact on postnatal growth and metabolic homeostasis is currently being examined.

Disclosure: Was this work supported by industry? No.

H-06
Differential effects of maternal nutrient restriction during early—mid-gestation on renal glucocorticoid and angiotensin ii receptors mRNA abundance when followed by juvenile obesity

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Maternal nutrient restriction (NR) in early—mid-gestation results increased renal glucocorticoid sensitivity in offspring raised under optimal conditions. This study was conducted to examine the effect of raising previously nutrient-restricted (NR) offspring within a sedentary hyperphagic physical environment on renal glucocorticoid receptor (GR), angiotensin II type 1 and type 2 receptor (AT1, AT2) mRNA abundance.
Pregnant sheep were randomly allocated to a control (C, 7 MJ/day, $n=7$) or a NR diet (50% of C, $n=11$) from 30 until 80 days gestation, thereafter receiving 100% calculated metabolisable energy requirements. Offspring were reared by their mothers to weaning and thereafter, from 4 to 12 months of age, in a restricted activity environment with ad libitum access to food, to promote obesity. Following humane euthanasia, renal GR, AT1, AT2 mRNA, and 18S rRNA abundances were quantified by qRT-PCR.
Whilst all animals were obese, mean body weights (C 91 ± 2, NR 88 ± 1 kg) were similar. Maternal NR significantly reduced GR (C 96.8 ± 18.0, NR $25.3 \pm 5.97 \times 10^{-4}$ relative to 18S rRNA ($p<0.0001$)) and AT1 (C 2.83 ± 0.521, NR $1.07 \pm 0.217 \times 10^{-6}$ to 18S ($p=0.001$)) mRNA abundances. Whilst AT2 mRNA abundance showed a similar trend, this did not reach statistical significance (C 3.59 ± 0.648, NR $1.72 \pm 0.366 \times 10^{-7}$ to 18S).
These findings contrast with those seen in offspring reared in an unrestricted activity environment, emphasising the importance of the juvenile environment in determining adverse outcomes following in utero exposure to nutrient restriction.

Disclosure: Was this work supported by industry? No.

H-07
Maternal nutrient restriction during early fetal development has differential effects on adiponectin, leptin, and tumour necrosis factor alpha mRNA expression in perirenal adipose tissue of adult offspring

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Adipose tissue is a major endocrine organ responsible for the release of hormones, termed adipokines. Of these, leptin and tumour necrosis factor (TNF) alpha increase with obesity, whilst adiponectin, which ordinarily reduces insulin resistance, decreases.
Singleton-male-bearing sheep were fed 100% of total metabolisable energy (ME) requirements (C: $n=8$) or 50% of this (NRE: $n=10$) up to 95 days of gestation. Thereafter, all consumed 100% of ME requirements up to term (147 days). PAT was sampled at 3 years of age. All procedures accorded with current UK legislation. Adiponectin, leptin and TNF alpha mRNA abundances were quantified by qPCR by the 2-deltaCT method with the housekeeping gene 18S as an internal control.
Offspring body weight control (C 75.6 ± 2.8, NR 75.0 ± 2.6 kg) and adiposity (C 16.8 ± 2.6, NR 19.4 ± 3.4 g/kg) to 3 years of age were unaffected by maternal nutrient restric-

tion. Although there were no differences between groups in the mRNA abundances of leptin (C 3.0 ± 2.3, NR 4.4 ± 2.8 ($\times 10^{-6}$)) or TNF alpha (C 12 ± 3.7, NR 6.6 ± 3.3 ($\times 10^{-5}$)), adiponectin (C 5.5 ± 1.2, NR 3.2 ± 1.2 ($\times 10^{-2}$), $p < 0.05$) mRNA was significantly upregulated in NR offspring.
In conclusion, although maternal nutrient restriction from 0 to 95 days gestation had no effect upon adult body weight or adiposity, upregulation of adiponectin mRNA expression may reflect a compensatory mechanism for adult offspring following maternal nutrient restriction in early gestation.

Disclosure: Was this work supported by industry? No.

H-08
Sex and twinning influence early gestation undernutrition effects on sheep offspring growth

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Objectives: Multiple pregnancy affects size at birth and growth pattern from as early as 8 weeks gestation (Iffy et al., 1983. Am. J. Obstet. Gynecol. 146, 970—972). Male embryos grow at a greater rate than females (Pedersen, 1980. Br. Med. J. 281, 1253). We hypothesised that moderate maternal undernutrion in early gestation will have a greater effect on male offspring growth, particularly if combined with the increased constraint of being a twin.
Methods: Welsh Mountain ewes received 100% (C, $n = 41$) or 50% nutrient requirements (U, $n = 47$) from 1 to 31 days gestation (dGA), and 100% thereafter. Ewes were weighed weekly and blood samples were collected at − 1, 30, and 65 dGA for cortisol analysis (Immulite analyser, DPC).
Results: At day 31, U ewes had gained less weight than C ewes and had a lower plasma cortisol concentration ($p < 0.05$). During 1—31 dGA, twin bearing ewes gained less weight than singleton bearing ewes. At birth, twins were smaller than singleton lambs ($p < 0.05$). Weight gained between birth and 12 weeks old and weight at 12 weeks old were greater in U males compared to C males, an effect that was predominantly in twins ($p < 0.01$). Data were analysed by ANOVA.
Conclusion: The increased constraint of being a twin and a male embryo in a nutrient-restricted intrauterine environment induces a phenotype more likely to gain weight in a good postnatal environment.
Supported by the British Heart Foundation.

Disclosure: Was this work supported by industry? No.

H-09
Association between maternal seafood consumption before pregnancy and fetal growth, evidence for an association in overweight women. Results from the French EDEN Study (study of pre- and early postnatal determinants of the child's development and health)

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Recent studies, in countries with high seafood consumption, suggest its benefit on fetal growth and infant development.
Objectives: To determine the association between seafood consumption in French pregnant women and fetal growth.
Subjects and methods: Pregnant women were invited to participate into the study. Women ($n = 1629$) answered two food frequency questionnaires on their usual diet in the year prior pregnancy and during the last term of pregnancy. Fetal circumferences were measured by ultrasounds. Anthropometric data were measured at birth. Variables were compared across tertiles of the mother's seafood consumption by multiple linear regressions adjusted for centre, mother's age, smoking habits, height, parity, gestational age, and the newborn's sex. Due to significant interaction, analyses were stratified according to pre-pregnancy body mass index ($< \text{or} \geq 25$ kg/m^2).
Results: For overweight women ($n = 413$), a higher seafood consumption before pregnancy was associated with higher fetal biparietal and abdominal circumferences ($p < 0.05$), birth weight ($p < 0.0004$), and other anthropometric data ($p < 0.01$). From the lowest to the highest tertile, mean birth weight was 166 g higher ($p < 0.001$). Adjustments for the mother's educational level or cardiovascular and metabolic parameters did not change these results. No significant association was found with seafood consumption at the end of pregnancy.
Conclusion: High seafood consumption in early pregnancy either directly or indirectly sustains fetal growth in overweight women. Follow-up of the infants may help determine whether this has beneficial consequences for the child's health and development.

Disclosure: Was this work supported by industry? No.

H-10
Programmed increase in appetite and growth rate in juvenile microswine offspring of protein restricted sows

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Microswine offspring of maternal protein restriction (MPR) have low birth weights; body weights equal controls by adulthood. Our *objective* was to quantify the changes in rates of growth between normal- and low-protein offspring (NPO, LPO), and to determine whether changes in food intake contribute to altered juvenile growth patterns.
Methods: Time-mated sows were exposed to isocaloric MPR (1% vs. 14%) in last 1/3 of gestation +2 weeks (period of nephrogenesis). Weights and ad lib food intake (g/kg current wt/meal) were measured in offspring on 3—7 days/week over 6 to 13 weeks of age, all on normal piglet chow from week 2.
Results: LPO ($n = 15$, 0.90 kg) birth weights are reduced by 1.6 standard deviations (S.D.) vs. NPO ($n = 12$, 1.04 kg); nadir

weights at 2 weeks were further reduced by 4.0 S.D. LPO weights catch up to NPO at 9.9 weeks. Rate of growth (as weekly % increase in weight) shows a significant time x diet interaction: LPO grow more slowly than NPO from birth to 6 weeks, then maintain a higher rate of weight increase vs. NPO from 6 through 13 weeks (interaction, $p<0.0001$). Food intake is consistently increased in LPO vs. NPO over 6–13 weeks; averages pooled across weeks are 34.0 ± 1.7 g/kg/meal in LPO vs. 28.3 ± 3.3 in NPO, $p=0.0007$.
Conclusions: LPO exhibit a programmed increase in appetite and an increase in rate of growth from 6 weeks forward. Persistence of increased appetite and rate of weight gain ($\geq$13 weeks) beyond the point of catch-up (=10 weeks) suggests potential for lifelong energy imbalance.

Disclosure: Was this work supported by industry? No.

H-11
Does maternal size and feeding during pregnancy affect lean mass and bone content in sheep?

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Introduction: Little study has been directed toward the role of muscle forces in *in utero* bone development, and bone's response to them. The amount of lean mass (LM) is presumably related to muscle force. This paper presents data on bone mineral content (BMC) and LM in lambs at day_{140} of pregnancies exposed to different intrauterine factors.
Materials/methods: Five twin-bearing and 10 singleton-bearing Romney "small" (S) or "large" (L) ewes were fed either a high (H) or maintenance (M) amount of pasture diet from day_{21} of pregnancy, and were euthanased at day_{140}. Fetal hindquarters and caudal spine were scanned (DEXA). Mean absolute and corrected (to leg length) BMC and LM were analysed for differences between groups 1–4 (single/L/H {=single lambs born from large ewes fed the high diet}, twin/L/H, single/L/M, and twin/L/M, respectively) and groups 5–8 (single/S/H, twin/S/H, single/S/M, and twin/S/M, respectively), $n=10$/group.
Results: Corrected LM and BMC were significantly less in twin than singleton groups ($p=0.001$). There were significant between-group differences in BMC/LM ratio. For instance, groups 1 and 5 were not significantly different ($p=0.154$) but were significantly different from others ($p=0.001$–0.002); groups 6, 7, and 8 were not different, but were from all other groups ($p=0.000$–0.045).
Conclusion: In lambs of similar genotype, a given amount of muscle was associated with variable bone mass, perhaps due to one or more of: maternal nutrition, maternal size, fetal size, placental function, and alteration of the set point of the bone mechanostat.

Disclosure: Was this work supported by industry? No.

H-12
Plasma adiponectin levels of high molecular weight form in newborns are higher than those in healthy children and positively correlated with total adiponectin

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Background: Adiponectin exists in human blood as three main forms; the trimer (LMW), hexamer (MMW), and high molecular weight (HMW). It has been shown that the HMW adiponectin is an active form. However, no information is available with respect to these three main forms in newborn infants and children. Our aim is to determine plasma levels of these three forms in newborns and children.
Study design: Blood samples were obtained from 32 healthy newborns (cord blood, 17 males and 15 females) and 22 healthy children (12 males and 10 females, 6–15 years). The total, HMW, and the combined HMW+MMW adiponectin concentrations were measured by ELISA. The MMW and LMW levels were calculated by these measurements.
Results: No gender difference among the study subjects was found in the total and three forms of adiponectin concentrations. There were significantly differences in the mean concentration of the total (17.5 μg/ml vs. 6.8 μg/ml, $p<0.001$), HMW (13.2 μg/ml vs. 3.6 μg/ml, $p<0.001$), and MMW adiponectin (2.7 μg/ml vs. 1.5 μg/ml, $p<0.001$) between the newborn infants and the healthy children. The ratio of the percentage of HMW form of adiponectin to total adiponectin in the newborn subjects was significantly higher than those in the children (72% vs. 50%, $p<0.001$). Three forms of adiponectin concentrations were closely correlated with the total adiponectin levels in the children, whereas only the HMW adiponectin levels were correlated with the total adiponectin levels in the newborn infants.
Conclusion: Plasma adiponectin in newborn infants mainly exists in the HMW form and its ratio to total adiponectin may decrease during postnatal life.

Disclosure: Was this work supported by industry? No.

H-13
Effect of a cholesterol-lowering diet during pregnancy on maternal and fetal Doppler velocimetry: the Carrdip study

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Objective: To study the effect of a low-cholesterol low saturated fat diet on Doppler indices in the fetus and mother.
Materials/methods: 290 nonsmoking Caucasian women, aged 21 to 38 years, without previous pregnancy complications and carrying a single fetus were randomized to continue their usual diet (controls, $n=149$) or to adopt a low-cholesterol low-saturated fat diet (intervention, $n=141$) from gestational weeks 17—20 to birth. Doppler velocimetry of the umbilical artery and both uterine arteries were assessed at gestational weeks 24, 30, and 36.
Results: The physiological decrease in umbilical artery pulsatility index (PI) from week 24 to week 30 was more pronounced in the intervention compared to the control group with median values (interquartile range) of -0.17 (-0.29, -0.06) and -0.11 (-0.25, 0.01), respectively ($p=0.048$). Assignment to the intervention diet did not influence the changes in mean PI value of the two uterine arteries ($p=0.3$). The change in umbilical artery PI and mean PI value of the uterine arteries between weeks 24 and 36 were not significantly different between the two groups ($p=1.0$ and $p=0.2$, respectively). The degree of physiological decrease in umbilical artery from weeks 24 to 30 was significantly correlated with the degree of increase in LDL cholesterol in the same period ($r=0.197$, $p=0.03$).
Conclusion: Our study shows that dietary intervention can modify fetoplacental circulation in midpregnancy. Specifically, a cholesterol-lowering diet during pregnancy may favorably affect Doppler velocimetry in the umbilical artery.

Disclosure: Was this work supported by industry? No.

H-14
Effect of late gestational feed restriction on intermediary metabolism in lambs

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Objective: This study aimed to evaluate ketone bodies metabolism during 3 days fasting in the offspring born from ewes fed with either adequate or restricted energy supply during late gestation.
Materials and methods: Seven-month-old lambs (10 F, 10 M) born from dams fed either 60 % (R, $n=14$) or 100% (A, $n=6$) of their energy requirements during the last trimester of gestation were fasted for 3 days. At 00, 48, 72 h of fasting, and 2 h after re-feeding, blood were sampled. Plasma concentration of glucose, insulin, β-OH-butyrate, non-esterified fatty acids (NEFA), and urea were determined.
Results: R-offspring had significantly higher plasma concentrations of β-OH-butyrate (6.56 ± 0.03 vs. 6.7 ± 0.02 log (μm/l), $p<0.001$) and lower NEFA (1.13 ± 0.05 vs. 0.99 ± 0.03 (mM/l), $p=0.02$) than A-offspring. Concentrations of insulin were slightly lower in R-offspring (4.7 ± 0.09 vs. 5.0 ± 0.15 log (ρg/l), $p=0.13$). No significant differences were found in plasma glucose (3.2 ± 0.07 vs. 3.2 ± 0.06 (mM/l)) and urea (9.5 ± 0.3 vs. 9.4 ± 0.2 (mM/l)) between the two groups. Females had higher β-OH-butyrate (6.67 ± 0.03 vs. 6.59 ± 0.03 log (μm/l), $p=0.04$) and lower urea (9.1 ± 0.2 vs. 9.8 ± 0.2 (mM/l), $p=0.02$) than males regardless of maternal nutrition and sampling time. Male had significantly ($p<0.05$) higher insulin than female except at 72 h of the fasting.
Conclusion: The results suggest that late gestational feed restriction can have long-term effects on ketone body metabolism in offspring.

Disclosure: Was this work supported by industry? No.

H-15
Fasting energy expenditure is more related to birth weight than to gestational nutrition in sheep

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Objective: Gestational feed restriction (GFR) often results in lower birth weight (BW). However, twins may not be equally affected. This study aimed to distinguish the effect of gestational nutrition vs. birth weight separately on fasting energy expenditure (FEE/$kg^{0.75}$) in twin lambs.
Materials and Methods: Seven-month-old lambs (10 F,10 M) born from dams fed either 60% (R, $n=14$) or 100% (A, $n=6$) of their energy requirements during last trimester of gestation were fasted for 3 days. During the last 24 h of fasting, O_2 consumption and CO_2 production were measured and FEE/$kg^{0.75}$ calculated (1). Data were analyzed using two models. Model 1: gender + GFR. Model 2: gender + BW (⟨mean⟩). Dam was a random effect in both models. Log BW was a covariate in model 1.
Result

Table 1 Effects of gestational nutrition or birth weight on FEE/$kg^{0.75}$ in growing twin lambs

Model	1-GFR		2-BW	
	Adequate	Restricted	> Mean	< Mean
Female	306 ± 21	330 ± 12	$311^{ab}\pm12$	$353^{a}\pm18$
Male	314 ± 18	307 ± 13	$327^{ab}\pm18$	$301^{b}\pm12$

Conclusion: FEE/$kg^{0.75}$ is more closely related to BW than to gestational nutrition. In low birth weight, male has lower FEE/$kg^{0.75}$ than females.
Ref. 1. Brouwer, E (1965). Energy metabolism of farm animals, pp. 441—443 [K.L. Blaxter, ed.]. Academic Press, London.

Disclosure: Was this work supported by industry? No.

H-16
GLP-1 and leptin secretion are modulated by an early postnatal high fiber diet

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Objective: There is a growing body of evidence suggesting risk of developing diseases such as diabetes and obesity may be programmed early in life. The objective of this study was

to examine the effects of early diets high in protein or fiber on satiety hormone expression and secretion as well as to identify potential critical periods for these genes and hormones.
Materials/methods: Virgin Wistar dams were mated and given access to standard chow and water ad libitum. At parturition, litters were culled to 10 pups. Pups were weaned at 21 days onto standard chow or a diet high in protein (40%) or fiber (30%). On days 7, 14, 21, 28, and 35 after birth, a male and female pup from each litter were anaesthetized. Organs were collected for mRNA expression analysis and blood drawn for analysis of satiety hormone secretion.
Results: The high fiber diet caused a significant increase in small intestine and colon length and weight when adjusted for body weight ($p \leq 0.05$). The high fiber diet caused significant increase in plasma GLP-1 ($p \leq 0.01$) and significant decrease in plasma leptin ($p \leq 0.05$). Independent of diet, a potential critical period for the secretion of GLP-1 was identified. Plasma levels of GLP-1 decreased significantly between 7 days and 21 days ($p \leq 0.01$) and then appeared to level off between 21 days and 35 days.
Conclusions: Preliminary data suggests that an early postnatal diet high in fiber may program the metabolic systems related to glucose and lipid metabolism and provide potential protection against the development of certain chronic diseases.

Disclosure: Was this work supported by industry? No.

H-17
Effects of maternal weight gain during the first trimester on fetal and placental growth

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Effects of maternal weight gain during the first trimester on fetal and placental growth

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Objective(s): The purpose of this study is to investigate the effect of the pattern of maternal weight gain in the three trimesters of pregnancy on fetal and placental growth.
Material/method(s): We used medical data on pregnancy course and outcome for deliveries occurring between 1988 and 2005 at the University of Tokyo. After excluding subjects with preterm labor, multiple gestation, preeclampsia, diabetes mellitus, asthma, a history of habitual abortion or infertility, uterine fibroid, endometriosis, a habit of smoking, fetal anomalies, fetal distress, or any other complications, 209 cases including 116 cases with SGA ($< -3/2$ S.D.) infants and 93 cases with AGA infants were recruited. Maternal weight gain during each trimester was calculated according to the Institute of Medicine guidelines and classified into the two groups: low and high weight gain.
Result(s): Maternal weight gain in the first trimester had no significant effect on fetal and placental weight. However, in the cases with high weight gain in the first trimester, low weight gain in the second trimester showed a significant decrease in placental weight than high weight gain. In the cases with high weight gain in both the first and second trimester, low weight gain in the third trimester showed a significant decrease in fetal weight than high weight gain.
Conclusion(s): This study suggested that high maternal weight gain in the first trimester may increase the risk of fetal and placental growth restriction, if it is followed by low weight gain in the second or third trimester.

Disclosure: Was this work supported by industry? No.

H-18
The efficacy of estrogen replacement therapy on vascular dysfunction to bradykinin induced by maternal low protein diet

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Objectives: Our previous study showed that estrogen played an important role in limiting cardiovascular dysfunction induced by maternal protein restriction. Therefore, we hypothesized that estrogen replacement therapy (ERT) would improve the cardiovascular dysfunction of ovariectomized offspring induced by a maternal protein restriction.
Methods: Wistar rats were fed a diet containing either 18% (control group, C) or 9% casein (protein restriction group, R) throughout pregnancy only. On day 50, the offspring in the C and R groups were divided into three groups each, and the intended operation was performed (sham group (CO, RO), ovariectomized group (CX, RX), ovariectomized, and ERT group (CE, RE). On day 175, the offspring were killed and the mesenteric and renal arteries dissected. Endothelial function was investigated with a wire myograph in both arteries.
Results: Mesenteric artery: The vasodilatory response to bradykinin (BK) tended to attenuate in RX, and ERT made this attenuation improve. The vasodilatory response to sodium nitroprusside (SNP) was not different between the four groups (CX, RX, CE, RE). Renal artery: The response to BK tended to attenuate as well. However, ERT was not effective in improving it. The response to SNP was significantly attenuated in the R groups and was improved by ERT.
Conclusions: It was revealed that NO production through BK in endothelial cells was attenuated in both arteries by maternal protein restriction. ERT can be considered to have induced improvement of NO production and NO sensitivity. It is likely that there was a difference in the efficacy of ERT to NO production through the BK receptor on endothelial cells and NO sensitivity on smooth muscle cells between the peripheral and large arteries.

Disclosure: Was this work supported by industry? No.

H-19

Does maternal diet induced obesity (DIO) program the development of the metabolic syndrome in the (adult) offspring?

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With the current obesity epidemic, pre-gravid obesity is more and more common so that more and more pregnant women are also obese. Pre-pregnancy obesity has been associated with pregnancy and delivery complications. Moreover, several epidemiological studies have suggested a correlation between pre-pregnancy maternal body mass index and increased risk of obesity in children and young adults.
The aim of our current studies is therefore to assess how maternal obesity could "program" the development of the metabolic syndrome, and especially obesity and insulin resistance, in the offspring. We developed a model in which female rats were rendered obese by consuming, from weaning on, diets enriched in fat and/or sucrose (OB1: 45% kcal fat, 15.5% kcal sucrose, and OB2: 45% kcal fat + sweetened condensed milk). A group of control females fed lab chow was also included. At 3 months of age, obese and control females were mated, and allowed to deliver spontaneously. Litter size was culled to eight pups. Dams were fed their usual diets during lactation. Offspring were weaned onto the control diet at 21 days of age.
Our results show that the offspring of the obese dams had a lower birth weight (OB1: 6.2 ± 0.3 g, OB2: 6.1 ± 0.1 g vs. control: 6.8 ± 0.1, ANOVA $p < 0.0001$). Afterwards, the offspring of the OB1 and OB2 mothers presented an increased weight gain, so that at 3 months of age, both male and female OB1 and OB2 offspring were significantly heavier.
Ongoing work includes the assessment of the offspring's insulin sensitivity by the hyperinsulinemic—euglycemic clamp technique. *(Supported by EARNEST, Food-CT-2005-007036)*.

Disclosure: Was this work supported by industry? No.

H-20

Paradoxically increased placental leptin mRNA expression in response to maternal food restriction

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Objective: Leptin, a mediator of fetal growth and appetite development, is transported by the short isoform receptor (Ob-Ra) and acts via its long isoform receptor (Ob-Rb). Placental expression of these receptors may mediate leptin actions within the placenta or regulate transport of maternal, placental and/or fetal leptin. Maternal food restriction (MFR) causes growth retardation and hypoleptinemia in newborns. We determined the impact of MFR on maternal plasma leptin and placental expression of leptin, Ob-Ra and Ob-Rb.
Materials/methods: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy days 10 to 16. At e16, maternal plasma leptin levels and placental mRNA expression of leptin, Ob-Rb and Ob-Ra were determined. mRNA data is normalized to β-actin and expressed as deltaCT (higher amounts correspond to lower CT).
Results: MFR dams had significantly lower body weight gain with decreased plasma leptin levels (0.7 ± 0.1 vs. 1.4 ± 0.2 ng/ml, $p < 0.05$) as compared to control dams. Placental mRNA expression of leptin was significantly increased (10.1 ± 0.2 vs. 12.5 ± 0.3 CT, $p < 0.01$), whereas placental Ob-Rb (11.9 ± 0.3 vs. 9.4 ± 0.2 CT, $p < 0.01$) and Ob-Ra (22.7 ± 0.4 vs. 19.4 ± 0.3 CT, $p < 0.001$) was significantly reduced in MFR pregnancies.
Conclusions: MFR results in decreased maternal plasma leptin levels, likely a result of reduced body fat. The decrease in placental expression of Ob-Rb and Ob-Ra may contribute to reduced fetal growth and alter the development of anorexigenic vs. orexigenic mechanisms. We propose that increased placental leptin expression represents an intrinsic response to augment placental growth and maternal—fetal nutrient transfer.

Disclosure: Was this work supported by industry? No.

H-21

Effects of twinning and perconceptional undernutrition on the HPA axis of the late gestation sheep fetus

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Objectives: Altered function of the hypothalamic—pituitary—adrenal axis (HPAA) is postulated as a key mechanism underlying the developmental origins of adult disease. In humans, birth weight is inversely related to fasting cortisol levels in adulthood. In experimental animals, exogenous glucocorticoid administration, periconceptional undernutrition, and placental insufficiency have all been shown to alter the fetal and postnatal HPAA. Twins are smaller at birth than singletons but little is known about the effect of twinning on HPAA function. Our objective was to assess the effects of twinning and periconceptional undernutrition on HPAA function in late gestation.
Methods: Ewes were well-fed (7 singleton-bearing, 8 twin-bearing) or undernourished (UN) from 60 days before mating until 30 days after (12 singleton-bearing, 8 twin-bearing). All fetuses were catheterised and underwent a combined CRH and AVP challenge on day 127 of gestation, and a metyrapone challenge on day 128.
Results: The ACTH area under the curve (AUC) response to the CRH/AVP challenge is greater in twins than singletons (33.6 ± 2.7 vs. 23.8 ± 3.6 ng min ml^{-1}, $p = 0.04$), but cortisol response was not different. Similarly in the metyrapone challenge, the ACTH response to the metyrapone-induced cortisol decrease was greater in twins than in singletons (0.90 ± 0.15 vs. 0.36 ± 0.19 ng min ml^{-1}, $p = 0.03$). Undernutrition had no effect on the AUC responses.

Conclusion: These data suggest that increased central sensitivity of the HPAA in twins, also demonstrated in postnatal lambs, is already present before birth.

Disclosure: Was this work supported by industry? No.

H-22

'Malprogramming' of the hypothalamic melanocortinergic system in neonatally overfed rats

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Objective: Early postnatal overnutrition predisposes to obesity and diabetes in later life. Neuropeptides expressed in the arcuate hypothalamic nucleus (ARC) are involved in the regulation of food intake and body weight. Expression of orexigenic neuropeptide Y (NPY) and anorexigenic alpha-melanocyte-stimulating hormone, a post-translational cleavage product of proopiomelanocortin (POMC), is modulated by circulating leptin and insulin via leptin receptors (Ob-R) and insulin receptors (Ins-R). Early postnatally overnourished rats showed hyperleptinaemia and hyperinsulinaemia but no decrease in NPY expression in the ARC (J. Neuroendocrinol. 1999; 11:541). We investigated whether early postnatal overnutrition might also affect the melanocortinergic system.
Materials/methods: To induce early postnatal overnutrition, the primary litter size of Wistar rats was reduced from day 3 to day 21 of life to only 3 pups per mother (small litters, SL). Expression of neuropeptides and receptors was determined in ARC micropunches on day 21 of life by RT-PCR.
Results: 21-day-old SL rats showed overweight, hyperleptinaemia, and hyperinsulinaemia ($p<0.001$). Expression of Ob-R and Ins-R did not differ between groups. However, expression of the long form of the leptin receptor (Ob-Rb) was downregulated in SL rats ($p<0.05$), while POMC-expression was not upregulated despite hyperleptinaemia and hyperinsulinaemia.
Conclusions: These results indicate a 'malprogramming' of the hypothalamic melanocortinergic system, which might contribute to permanent overweight and associated diabetic disturbances after early postnatal overnutrition. *Supported by DFG (PL 241/4-1).*

Disclosure: Was this work supported by industry? No.

H-23

Mice with perinatal essential fatty acid deficiency have low leptin levels and low body fat as adults

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In previous studies, we have shown that essential fatty acid deficiency (EFAD) in perinatal period results in low body weigh and leptin levels in rat pups, but high body weight and normalised leptin levels in adult rats. The aim of this study was to study perinatal EFAD in mice and measure leptin levels, body weight, and body composition in the adult offspring.
Mice of C57BL/6 strain were given EFAD or control diet in late pregnancy and during lactation. All 3-week-old pups were weaned to ordinary chow and the body weight was recorded every week. From 15 weeks of age, one group of EFAD mice and one group of control mice were fed high fat diet. Plasma leptin was measured by ELISA at 3, 8, 15, and 20 weeks of age. The body composition was measured by dual energy X-ray absorption at 19 weeks of age.
Leptin levels were lower in the EFAD pups compared to controls at all ages. The EFAD offspring had lower body weight than the controls already at 1 week of age and the weight difference persisted up to adult age. The adult male EFAD mice and female EFAD mice fed high-fat diet had lower percentage body fat compared to controls, while the body fat did not differ between female groups on standard diet.
Perinatal EFAD in mice results in growth retardation, lower leptin levels and lower body fat in adult age. Leptin levels in early age might be of importance for later fat accumulation.

Disclosure: Was this work supported by industry? No.

H-24

Perceived barriers to the initiation of breastfeeding with a view to improving breastfeeding rates in Ireland

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Objectives: To describe the barriers to breastfeeding initiation and examine the reasons why mothers choose to formula feed in Ireland, a country with the lowest breastfeeding rate in Europe.
Materials and methods: A cross-sectional prospective study involved the recruitment of 309 pregnant women with the subsequent follow-up of mothers at 6 weeks and 6 months post-partum. Quantitative data was recorded detailing mothers' infant feeding decisions and perceived barriers to breastfeeding initiation.
Results: 59% of mothers were formula feeding upon hospital discharge (mean 3.7 days). Of these mothers, the principal reasons cited for choosing to formula feed included practicality/convenience (22%), not wanting to breastfeed (13%), and the lifestyle limitations associated with breastfeeding (12%). The main barriers to initiation included embarrassment of breastfeeding in public (25%), lifestyle limitations (19%), and negative exposure to breastfeeding (9%) along with feelings of incompetence in being able to successfully breastfeed (7%).
Conclusion: Results suggest that in order to increase breastfeeding rates in Ireland, the practice must be seen as a 'cultural norm'. Public health initiatives should focus on improving the public perception of breastfeeding and aim to instil confidence in women that the practice is achievable for all mothers.

Disclosure: Was this work supported by industry? No.

H-25
Why mothers stop breastfeeding during the first 6 months of life?

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Objectives: To identify those factors that influence mother's decision to discontinue breastfeeding practice prematurely during the first 6 months of life.
Materials and methods: A cross-sectional prospective study involved the recruitment of 309 pregnant women with subsequent follow-up at 6 weeks and 6 months post-partum. Quantitative data was recorded detailing mothers' infant feeding practices and reasons for early discontinuation of breastfeeding.
Results: While 41% of women were breastfeeding upon discharge from hospital, only 28% of mothers were still breastfeeding at 6 weeks post-partum. The main barriers to successful continuation included tiredness post-birth (23%), physical complications (22%), and frequent feeding routine (20%). Only 14% of mothers were still breastfeeding at 6 months; main reasons cited for discontinuation of breastfeeding from 6 weeks to 6 months included frequent feeding routine/increased demands on mother (36%), work return in 16% of cases, while 15% of mothers discontinued when they felt they were ready/had enough.
Conclusion: Increased demands/on-demand feeding was identified as a common attitudinal barrier to continued breastfeeding during the first 6 weeks and 6 months of life. Work return was highlighted as a negative determinant of breastfeeding duration suggesting the need to increase workplace flexibility for breastfeeding mothers.

Disclosure: Was this work supported by industry? No.

H-26
Effect of fetal growth restriction on body composition and hormonal status at birth in infants of low and appropriate for gestational age birth weight

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The aim of the study was to evaluate the impact of intrauterine growth restriction (IUGR) on body composition and hormonal status in newborns of small (SGA) and appropriate-for-gestational age (AGA) birth weight.
Fetal growth was evaluated by 4 ultrasound examinations in 145 women. Intrauterine growth slope (IUGS) was calculated as fetal weight percentiles change and IUGR defined as its reduction by more than 20 percentiles. SGA was defined as birth weight below the 10th percentile. Newborns were stratified into four groups: SGA-IUGR ($n=27$), 'constitutional' SGA (SGA-C, $n=21$), AGA-IUGR ($n=42$), and 'constitutional' AGA (AGA-C, $n=55$). Cord insulin and IGF-I concentrations were determined. DEXA scan was performed in newborns at day 3.
Fat mass percent was similar between SGA-IUGR and SGA-C newborns ($14.9\pm6.2\%$ vs. $15.2\pm6.8\%$, $p=0.9$), but significantly lower in AGA-IUGR vs. AGA-C group ($17.0\pm6.5\%$ vs. $19.8\pm6.6\%$, $p=0.01$). Insulin concentration was lowest in SGA-IUGR and highest in AGA-C group, but the difference failed to reach statistical significance. IGF-I levels were decreased in SGA-IUGR vs. SGA-C and in AGA-IUGR vs. AGA-C groups (28.5 ± 16.5 vs. 43.9 ± 26.8 ng/ml, $p=0.009$ and 52.8 ± 29.8 vs. 65.4 ± 26.3 ng/ml, $p=0.02$). In multivariate analysis, birth weight was directly related to IUGS and cord IGF-I, and infant's fat mass to IUGS, cord insulin, and IGF-I concentrations.
In conclusion, IUGR affects body composition and hormonal parameters even in newborns of normal birth weight.

Disclosure: Was this work supported by industry? Yes: Pfizer.
Do you act as a consultant, employee or shareholder with this industry? No.

J. DIABETES, OBESITAS, METABOLIC SYNDROME

J-01
No decreased insulin sensitivity found in SGA preterm infants before 6 months corrected age

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Being small for gestational age (SGA) is a risk factor for decreased insulin sensitivity. Decreased insulin sensitivity has been shown already in 1-year-old infants showing rapid catch-up growth.
Objectives: (1) Do preterm SGA infants have decreased insulin sensitivity compared to preterm appropriate for gestational age (AGA) infants between 0 and 6 months corrected age. (2) Do preterm infants with postnatal growth retardation (PGR) have decreased insulin sensitivity.
Methods: Fasting insulin and glucose levels were measured at 0, 3, and 6 months corrected age in 83 infants (BW $\leq$1500 g or GA $\leq$32 weeks). Infants were categorized as SGA (birth weight $<P_{10}$, $n=16$), PGR (AGA at birth, weight at term $<P_{10}$, $n=14$), or AGA (weight at birth and term $>P_{10}$, $n=53$). Catch-up growth was defined as >0.67 S.D. weight increase between term and 6 m.
Results: Fasting glucose and insulin were significantly lower, whereas glucose/insulin ratio was significantly higher in SGA infants compared to AGA and PGR infants at 0 m (3.4 ± 0.7/12.7 ± 14.4 vs. 4.1 ± 0.7/22.5 ± 22.2 vs. 4.4 ± 0.7/23.2 ± 17.4 (mean $\pm$ S.D., glucose (mmol/l)/insulin (pmol/l)) but not at 3 and 6 m. No differences were seen between AGA and PGR infants or between SGA infants with or without catch-up growth.
Conclusion: Compared to AGA, SGA preterm infants are more insulin sensitive at 0 m. Although this insulin sensitivity decreases after term, fasting insulin, and glucose levels of SGA infants are not significantly different to those of AGA infants at 6 m. PGR did not influence insulin sensitivity.

Disclosure: Was this work supported by industry? Yes: Friesland Foods.
Do you act as a consultant, employee or shareholder with this industry? No.

J-02
Exposure to a "junk food" diet during gestation and lactation impairs skeletal muscle development, promotes adiposity, and influences feeding behavior in offspring

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We have developed a high fat/high sugar palatable cafeteria diet (CD) model to examine the potential fetal programming effects of a "junk food" diet on skeletal muscle and adipose tissue development as well as feeding behavior in offspring.
Rats were fed the CD during gestation alone (CDG) or during gestation and lactation (CDW) and compared with a control group fed standard chow (C). From weaning, the offspring were fed either CD or chow up to 10 weeks of age.
Weanling offspring from the CDW group exhibited increased adiposity characterized by adipocyte hypertrophy but not hyperplasia as well as intramuscular lipid accumulation, a sign of metabolic disruption. They also exhibited a 25% decrease in *semitendinosus* muscle cross sectional area with fewer fibers (and nuclei) compared with controls. This was accompanied by increased muscle PPARã, IGF-1, and IGF-1R mRNAs which might explain the intramuscular lipid deposition and an attempt to maintain insulin sensitivity. Rehabilitation to chow during lactation prevented adiposity but did not restore muscle fiber hyperplasia, suggesting irreversibly impaired muscle development and possibly function. The CDG offspring also exhibited reduced muscle cell proliferation (PCNA) and possible reduced glucose uptake via downregulation of insulin receptor and glut4 mRNAs.
Therefore, exposure to a "junk food" diet during pregnancy alone or during pregnancy and lactation impairs skeletal muscle development and metabolism; those detrimental effects are observable as early as the end of lactation. An analysis of the feeding behavior of mothers and offspring is ongoing and will also be presented at the meeting.

Disclosure: Was this work supported by industry? No.

J-03
Betel-chewing and trans-generational transmission of metabolic syndrome risk

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Objectives: To determine whether betel [*Areca catechu*] chewing, the fourth most common 'habit', relates to metabolic syndrome risk in children of betel chewing fathers since 5 days betel-feeding induces permanent obesity and hyperglycaemia in ~ 20% of non-betel-fed F1—F4 offspring of male CD1 mice, independent of F0 glycemia.

Materials/methods: Data was examined [multiple logistic regression analysis] from 5037 parent-child trios, identified from 10,566 families screened in Taiwan [the Keelung community-based screening program] for metabolic syndrome [NCEP ATP111] and its risk factors, for associations with betel-chewing, introduced in Taiwan in the 1940s and found to have be dose related to increases in risk of T2DM in betel-chewers.
Results: Paternal betel-chewing was independently associated with dose-related increases in early onset metabolic syndrome risk [and for each component individually] in their children. In offspring of parents without features of the metabolic syndrome, the risk ratio for non-chewing offspring was 2.53 [95% CI: 1.03—2.64] for paternal betel-chewers vs. never-chewers. The transmission effect was weakest for hyperglycemia and strongest for decreases in HDL-cholesterol.
Conclusions: The findings suggest that betel-chewing may induce metabolic dysfunction in adults and genetic or epigenetic changes in germ cells in men, independent of abnormalities contributing to the metabolic syndrome. Habit cessation could reduce metabolic syndrome related disease in future generations.

Disclosure: Was this work supported by industry? No.

J-04
Mismatched prenatal and post-weaning diets lead to sex-specific increases in fat deposition efficiency and exacerbates cardiovascular dysfunction in adult mouse offspring

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Objectives: We examined the effects of feeding a high-fat diet on food intake, growth, body fat mass and blood pressure in mouse offspring previously exposed *in-utero* to dietary protein restriction.
Methods: Female MF1 mice were fed isocaloric diets containing either 18% protein (C, $n=11$) or 9% protein (PR, $n=10$) during pregnancy. Weaned offspring were fed either a high-fat diet (HF, 45% kcal fat) or standard chow (SC, 21% kcal fat) until adulthood. Food intake, body weight, and blood pressure were monitored during this period. Offspring were killed at 16 weeks, and fat depots dissected and weighed.
Results: Similar growth patterns were observed in the C and PR groups on either HF or SC diet, although the HF offspring were heavier than the SC groups. Body fat mass increased two-fold ($p<0.001$) in all HF offspring. However, in PR-HF males, but not in females, daily energy intake was reduced by 20% compared to the PR-SC group ($p<0.001$). Systolic blood pressure in PR was greater by 16% and 10% in males and females vs. C ($p<0.05$), and increased further ($p<0.05$) by 15% and 7% in the HF male and female offspring, respectively.
Conclusions: Our results suggest that mismatched prenatal and post-weaning diets lead to sex-specific differences in fat deposition. This further exacerbates cardiovascular dysfunction in the adult offspring. The effects may be due

to changes in metabolic efficiency, and hence propensity to deposit fat, induced in prenatal life to give adaptive advantage in a predicted postnatal environment.
Supported by NIH and BHF.

Disclosure: Was this work supported by industry? No.

J-05
Maternal glucose tolerance during pregnancy: association with the newborn's weight and fat mass at birth. The EDEN Pregnancy and Birth-cohort Study

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Objective: The relationship between mother's diabetes during pregnancy and newborn anthropometry has been described but little is know about the relationship for the whole range of plasma glucose concentration in a general population of women.
Methods: The EDEN Study was proposed to all pregnant women visiting before the 22nd week of gestation in two maternities. Between 22 and 26 weeks of gestation, plasma glucose was measured 1 h after a 50 g oral glucose load (G1). The newborn anthropometry was measured 3 days after delivery. Newborn anthropometry was analyzed in association with G1 (divided by the following quintiles: 5.3, 6.0, 6.8, 7.6 mM) for the 722 women first included (of a total of 2007) in a regression model allowing adjustment for center, mother's age, gestational age, and newborn gender, and then additionally for mother's pre-pregnancy body mass index (pBMI).
Results: Mean birth weight increased steadily with G1 quintiles ($p=0.03$). The number of babies with a birth weight greater than 4000 g was respectively: 4.2, 3.5, 5.8, 7.0, 12.6 %1 ($p=0.02$). There was a strong association between G1 and the newborn sum of skinfolds ($p<0.003$) or wrist circumference ($p<0.007$). Those associations were not notably modified by additional adjustment for pBMI.
Conclusion: Maternal post-challenge glycaemia during pregnancy is associated with fetal growth over the whole range of its distribution. The association appears independent of pre-pregnancy weight. The follow-up of the children will permit further investigation on the relations between mother's glycaemia during pregnancy and postnatal health and development.

Disclosure: Was this work supported by industry? No.

J-06
Differences in body mass index and waist circumference in young women

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Objectives: Body composition marks the ability to function metabolically and in women has implications for fetal nutrient delivery and the risk of hypertensive disorders during pregnancy. Body mass index (BMI) is widely used to reflect body composition, specifically total adiposity, but recent studies of cardio-metabolic disease suggest that waist circumference (WC), a marker of fat distribution, is more closely linked to metabolic function. Accordingly, higher maternal BMI relates to increased risk of gestational hypertension but not pre-eclampsia, while greater WC relates to increased risk of both. We sought to determine whether high BMI and greater WC occur in the same or different women.
Methods: We measured height, weight, WC, and four skinfold thicknesses in 11,644 white Caucasian non-pregnant women living in Southampton, UK aged 20—34 years. We estimated percentage fat from skinfolds.
Results: 901 (8%) women had a BMI <25 kg/m^2 and a WC $\geq$ 80 cm; 749 (6%) women had a BMI ≥ 25 kg/m^2 and a WC <80 cm; 4108 (35%) had both a BMI ≥ 25 kg/m^2 and a WC ≥ 80 cm. Mean percentage fat in these three groups was 31.5, 32.5, and 37.0, and subscapular to triceps skinfold ratio was 0.95, 0.88, and 1.1, respectively.
Conclusion: Many women who are overweight do not have a high WC, and many who have a high WC are not overweight. Relations between anthropometric markers of a woman's body composition and her cardio-metabolic and reproductive function need to be carefully defined to further our understanding of maternal influences on pregnancy outcome.

Disclosure: Was this work supported by industry? No.

J-07
Programmed central adiposity and increased rate of fat accrual in juvenile microswine offspring of protein restricted sows

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Microswine offspring of maternal protein restriction (MPR) exhibit early growth restriction and full catch-up growth by adulthood. Our *objectives* were to assess effects of maternal diet and sex on fat and lean mass accrual and their distribution during the period of catch-up growth.
Methods: Time-mated sows were exposed to isocaloric MPR (1% vs. 14%) in last 1/3 of gestation + 2 weeks. Normal- and low-protein offspring (NPO, LPO) were scanned postnatally by dual energy X-ray absorptiometry at 6 and 11 weeks.
Results: At 6 weeks, LPO had reduced % body fat ($12.3 \pm 2.0\%$, $n=16$) vs. NPO ($14.8 \pm 2.4\%$, $n=12$, $p=0.003$); females ($n=11$) had higher % body fat vs. males ($n=17$, $p=0.012$). By 11 weeks, central adiposity (ratio of truncal/total body fat) was increased in LPO (0.9074 ± 0.015, $n=12$)

vs. NPO (0.8860 ± 0.014, $n=12$, $p<0.005$) as was the absolute increase in fat mass over 6–11 weeks, $p<0.005$. Again at 11 weeks, females ($n=10$) had elevated % body fat vs. males ($n=11$), $p=0.001$. Lean mass relative to length at 6 weeks was reduced in LPO vs. NPO ($p=0.001$) with no sex differences; by 11 weeks, it was similar in LPO and NPO, and reduced in females vs. males ($p=0.0047$).
Conclusions: Microswine LPO have reduced body fat and lean mass in early postnatal development. LPO on adlib diet after weaning accrue more fat than controls and show increased central adiposity by 11 weeks. Programmed increased in rate of fat accrual and its central location may result in progressive adiposity and metabolic dysfunction in adulthood.

Disclosure: Was this work supported by industry? No.

J-08
Statins during pregnancy and lactation in mice on hypercholesterolaemic diet prevents obesity, hypertension, and sedentary behaviour in adult offspring

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Objectives: We determined whether HMG-Co-A reductase inhibition during pregnancy and lactation in hypercholesterolemic mothers could prevent adverse phenotype development in their offspring.
Methods: Female C57BL/6 mice were fed either a high fat-high cholesterol diet (HF, 45% kcal fat) or standard chow (C, 21% kcal fat) from weaning through to pregnancy and lactation. Half of the pregnant mothers ($n=4$/group) were given an HMG-Co-A reductase inhibitor (pravastatin, 5 mg/kg/day) in the drinking water from the second half of pregnancy to weaning. Weaned offspring from each group were then fed either HF or C diets to adulthood. Body weight, blood pressure, and locomotor activity were assessed at 8, 9, and 10 weeks old.
Results: At 8 weeks old, offspring from HF mothers were significantly fatter (body weight, 14.9 ± 0.2 vs. 9.1 ± 0.5 g, $p<0.01$), hypertensive (systolic BP, 135.8 ± 1.9 vs. 101.4 ± 1.1 mm Hg, $p<0.01$) and less active (distance travelled, 321.8 ± 5.5 vs. 571.8 ± 11.6 cm, $p<0.01$) than offspring from C mothers independent of their postnatal nutrition ($p=0.01$ for all group comparison by ANOVA). Statin therapy in HF mothers reduced these effects in offspring independent of post-weaning nutrition (body weight, 11.5 ± 0.3 and 10.1 ± 0.2 g; systolic BP, 122.7 ± 1.4 and 121.9 ± 0.6 mm Hg; distance travelled: 467.5 ± 6.9 and 407.2 ± 4.3 cm on HF and C switch diet, respectively; $p<0.01$). These differences were sustained at 9 and 10 weeks old.
Conclusion: These findings suggest that HMG-Co-A reductase inhibition in hypercholesterolemic mothers may alleviate the risk of developing adverse metabolic and cardiovascular disorders in the offspring later in life.

Disclosure: Was this work supported by industry? No.

J-09
Severe intrauterine growth restriction is associated with increased carbohydrate intake in young women

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Objective: In animal models, perinatal events can program feeding preferences in adulthood. Thus, the aim of this study was to verify the association between intrauterine growth restriction (IUGR) and macronutrient food preference in human adults.
Materials/methods: 2050 individuals born between 1978 and 1979 in Ribeirão Preto, Brazil, were evaluated at the age of 23/25 years. Total caloric intake and macronutrient composition of habitual food were obtained with a food frequency questionnaire. IUGR was defined by birth weight ratio (BWR = birth weight/mean weight for gestational age) and stratified as non-restricted (BWR ≥ 0.85), mildly restricted (BWR <0.85 and ≥ 0.75), and severely restricted (BWR <0.75). Two-way ANOVA followed by LSD was performed with interaction evaluation. The model was controlled for BMI, maternal and participant's smoking and income, and participant's physical activity.
Results: There were 1830 non-restricted (NR), 154 mildly restricted (MR) and 66 severely restricted (SR) individuals. In general, there were no differences in total calories consumption between the three groups. However, evaluation of the interaction between groups and gender showed that SR women ate less protein ($p=0.01$). It was found that SR women ate more carbohydrate ($p=0.041$), increasing the carbohydrate/protein ratio ($p=0.01$). There was no effect of IUGR on fat consumption.
Conclusion: SGR in women was associated with increased spontaneous total caloric intake due to increased carbohydrate consumption. The results suggest that IUGR can influence appetite modulation, increasing the preference for carbohydrates. The programming of food preference by IUGR may play a significant role in the development of metabolic diseases in adulthood.

Disclosure: Was this work supported by industry? No.

J-10
Birth weight and subsequent risk of type 2 diabetes: a meta-analysis

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Objective: The 'small baby syndrome hypothesis' suggests that an inverse linear relation exists between birth weight and risk of type 2 diabetes. If so, this may have wide-ranging

consequences for preventive medicine. We conducted a meta-analysis on the relation between birth weight and risk of type 2 diabetes.
Materials/methods: A systematic review was performed on studies that provided odds ratio (OR) and 95% confidence interval (95%CI), or data for calculations, of type 2 diabetes associated with birth weight. Dichotomous comparisons (random and fixed effects), meta-regression, influence analysis, and assessment of publication bias were performed.
Results: 14 studies involving a total of 132,180 individuals were identified. Low birth weight (<2500 g), compared to >2500 g, was associated with increased risk of type 2 diabetes (OR: 1.32, 95% CI: 1.06—1.64). High birth weight (>4000 g), compared to <4000 g, was associated with increased risk of type 2 diabetes to the same extend as low birth weight (OR: 1.27, 95% CI: 1.01—1.59). Pooled OR increased when normal birth weight (2500—4000 g) was used as reference (low birth weight: 1.47, 95% CI: 1.26—1.72; high birth weight: 1.36, 95% CI: 1.07—1.73). Meta-regression showed a U-shaped relation between birth weight and risk of type 2 diabetes.
Conclusions: A relation between birth weight and risk of type 2 diabetes in later life exists. However, it is not linear inverse, but U-shaped. High birth weight is associated with increased risk of type 2 diabetes to the same extent as low birth weight. More research is needed to characterize etiopathogenic mechanisms to enable causal strategies of primary prevention. Supported by DFG (PL 241/3-1, 3-2).

Disclosure: Was this work supported by industry? No.

J-11
The 'small baby syndrome' hypothesis revised: animal studies and a meta-analysis

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Objective: Research on intrauterine growth restriction (IUGR) and subsequent development of type 2 diabetes and the metabolic syndrome is a rapidly expanding field in diabetes research, since potential implications for primary diabetes prevention are enormous.
Materials/methods: We performed a large animal study as well as a complementary meta-analysis on one of the main models, namely uterine artery ligation in rats (Lig).
Results: Surprisingly, our study showed that Lig neither leads to IUGR nor to neonatal catch-up growth, the pathogenetic co-factor in humans, and not to a programming of adipogenic and diabetogenic risk. Meta-analysis revealed that the literature is dominated by studies in which Lig pups with IUGR were actively selected. Accordingly, publication bias is evident ($p = 0.007$). Nevertheless, only one study found a subsequently increased risk.
Conclusions: Altered placental perfusion—the main cause of IUGR in humans in western countries—neither leads to IUGR nor to neonatal catch-up growth in Lig offspring, i.e., to none of the etiological factors of the human 'small baby syndrome'. Appropriate animal models of IUGR through decreased placental flow remain therefore to be established to uncover the pathophysiological basis of the 'small baby syndrome'. This may lead to new strategies of primary prevention of diabetes, obesity, and the metabolic syndrome. Supported by DFG (PL 241/3-1,3-2).

Disclosure: Was this work supported by industry? No.

J-12
Follow-up of offspring born to women with type 1 diabetes mellitus: study design

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Introduction: In a nationwide study on the outcome of pregnancy in women with type 1 diabetes mellitus (T1DM), conducted in The Netherlands in 1999—2000, maternal, perinatal, and neonatal complications were increased compared to non-diabetic women, despite generally well-controlled glucose levels. Some studies, but not all, of offspring born to women with T1DM show negative long-term effects on development, such as an increased rate of childhood and adolescent obesity and impaired glucose tolerance, subtle neuropsychological dysfunction and sometimes lower intelligence quotients.
Objective: We aim to investigate the long-term effects of a type 1 diabetic pregnancy on the development of the offspring at school age.
Study design: Children born to women with T1DM who participated in the original study are invited to participate in this follow-up study. A maximum of 308 children will be included. All children are approximately 6.5 years of age when tested. A control group of 90 children of the same age will be selected from offspring of women without diabetes during pregnancy who delivered at our hospital.
Outcome: Neuropsychological development, cardiovascular risk factors, immune function, cardiac function, and neuroendocrine reactivity are measured. Furthermore, we will study the metabolomic state of these children. We will look for associations between development at school age and complicating factors during or after pregnancy to see which of these factors (high HbA1c, maternal hypoglycemia, high birth weight, neonatal hypoglycemia) are related to developmental outcome in the offspring.

Disclosure: Was this work supported by industry? No.

J-13
Mechanisms of programmed hypertriglyceridemia and obesity: lipogenic and lipolytic gene expression

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Objective: IUGR offspring exhibit newborn fat accumulation and hypertriglyceridemia as obese adults. We hypothesized that programmed expression of lipogenic vs. lipolytic

enzymes contribute to the development of obesity. The transcription factor sterol-regulatory-element-binding-protein1 (SREBP1) induces expression of enzymes which modulate fatty acid uptake and synthesis and thus adipocyte lipid accumulation. We determined the expression of SREBP1 and key adipose tissue enzymes involved in lipogenesis (fatty acid synthase, FAS) and lipolysis (extracellular lipoprotein lipase, LPL, and intracellular hormone sensitive lipase, HSL) in IUGR pups.
Materials/methods: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy days 10 to 21 to produce IUGR newborns. At 1 day of age, adipose tissue was analyzed for SREBP1, FAS, LPL, and HSL mRNA levels using real-time RT-PCR. Data of is presented as threshold cycle (1/CT*100), normalized to β-actin.
Results: SREB1 expression was similar in IUGR and control newborns. However, the mRNA expression of the FAS (5.6 ± 0.6 vs. 8.3 ± 0.5, $p < 0.01$) and HSL (9.1 ± 0.6 vs. 12.5 ± 0.5, $p < 0.01$), though not LPL, were significantly reduced in IUGR.
Conclusions: IUGR offspring show a propensity to fat accumulation due to reduction in intracellular lipolytic enzyme, HSL. Decreased FAS expression, if persistent, would promote elevated plasma triglycerides, a finding observed in adult IUGR offspring. The reduced HSL and FAS and lack of change in SREBP1 suggests a programmed downregulation of SREBP1-HSL/FAS transcription mechanisms in IUGR offspring.

Disclosure: Was this work supported by industry? No.

J-14
PPARy2-mediated adipogenesis programs obesity in intrauterine growth restricted newborns

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Objective: Maternal food restriction (MFR) in pregnancy results in IUGR newborns which develop adult obesity, with excess body fat and hypertrophic adipocytes. Although enhanced orexigenic mechanisms potentiate food intake and weight gain, we postulated that programmed alterations in adipocyte gene expression contribute to body fat accrual. PPARγ2 is a glucocorticoid-mediated transcription factor which promotes adipocyte differentiation, capable of hypertrophy and lipid storage. We hypothesized that MFR programs offspring obesity via glucocorticoid-PPARã2 enhanced adipocyte differentiation.
Materials/methods: Control dams received ad libitum food, whereas MFR were 50% food-restricted from pregnancy days 10 to 21 to produce IUGR newborns. At birth, litter size was culled and all pups were nursed by Control dams and weaned to ad libitum feed. At 1 day and 9 months of age, adipose mRNA levels were analyzed for PPARγ2 and glucocorticoid receptor.
Results: At 1 day of age, the IUGR pups showed a six-fold increase in PPARγ2 and a four-fold increase in glucocorticoid receptor mRNA expression. At 9 months of age, IUGR pups exhibited increased body fat with persistently increased adipocyte expression of PPARγ2 (3.2 ± 0.3 vs. 1.0 ± 0.1, $p < 0.001$) and glucocorticoid receptor (2.5 ± 0.2 vs. 1.0 ± 0.2, $p < 0.01$).
Conclusions: Upregulation of newborn PPARã2 enhances differentiation of preadipocytes and promotes hypertrophic adipocytes. We propose that excess glucocorticoid augments transcriptional mediated adipogenesis in IUGR offspring. In addition to programmed central appetite pathways, peripheral obese adipocyte phenotype contributes to programmed obesity.

Disclosure: Was this work supported by industry? No.

J-15
Explaining the diabetes epidemic in Chinese—preliminary evidence from Hong Kong as a natural experiment in epidemiological time

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Objective: To test Barker's thrifty genotype hypothesis that rapid improvements in childhood living conditions could generate an epidemic of diabetes.
Methods: Taking advantage of a natural experiment, we used a population-based cross-sectional study to assess how childhood conditions, proxied by height, were associated with diabetes in 2378 long-term Hong Kong Chinese residents who had grown up in environments at different epidemiological stages.
Results: The association between height and diabetes depended on epidemiological stage during childhood ($p = 0.02$), adjusted for age, sex, socioeconomic status, marital status, and lifestyle habits. Shorter subjects who had grown up in a location at an early epidemiological stage (pre-industrial Guangdong in China) and taller subjects who had grown up in an environment at a later epidemiological stage (industrial, developed) Hong Kong had similar diabetes risk; all others had higher diabetes risks. Compared to taller subjects raised in Hong Kong, taller subjects from Guangdong had an adjusted odds ratio (AOR) of diabetes of 1.79 [95% confidence interval (CI) 1.04 to 3.07] similar to shorter subjects raised in Hong Kong AOR 1.76 [95% CI 1.09 to 2.84]. Adjustment for obesity slightly attenuated these associations.
Conclusions: The current epidemic of diabetes in Chinese may be partially generated by the rapidly improving childhood conditions associated with economic development, consistent with Barker's hypothesis.

Disclosure: Was this work supported by industry? No.

J-16
Analysis of critical periods of childhood BMI development for late-adolescent adiposity (both BMI and percentage body fat) using standard multiple regression, structural equation modelling, and spline-based approaches

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Objectives: To investigate critical periods of childhood BMI development for late-adolescent adiposity.
Materials/methods: At age 17 years BMI (BMI_{17}) and percentage body fat ($\%BF_{17}$) measured in the 279 girls and 202 boys of the Stockholm Weight Development Study (SWEDES) and related to their annual BMI values (age 0—10 years) obtained from maternal and school healthcare registries. Analysis approaches: (i) standard multiple regression (MR) on (a) absolute BMI values and (b) BMI velocities (0—1, 1—3, 3—6, and 6—10 years); (ii) structural equation models (SEMs) using BMI velocities (velocities as before); (iii) subject-specific splines used to identify key features of BMI growth curves, related to outcomes through MR.
Results: (i) (a) Only latest BMI value (BMI_{10}) significant for both outcomes in both sexes, and (b) all BMI velocities significant predictors of both outcomes in both sexes. Results only based on subset of children due to missing data. (ii) Gender differences found regarding critical periods for both outcomes. For BMI_{17}, velocities age 0—1 and 3—6 years significant for both boys and girls, but velocity age 6—10 years only in boys. For $\%BF_{17}$, velocities age 0—1 and 3—6 years significant only in girls, but velocity age 6—10 years significant for both sexes. (iii) Both age and BMI at adiposity rebound (AR) strongly associated with both outcomes in both sexes.
Conclusions: Critical periods of childhood BMI for development of late-adolescent adiposity differ depending on gender and on measure (BMI or %BF) used. Alternative approaches to MR advantageous when high prevalence of missing data.

Disclosure: Was this work supported by industry? No.

J-17
Factors associated with overweight tracking in South Africans from early to late childhood

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Objectives: To identify factors associated with overweight (OW) tracking in South Africans from two/three (early childhood) to 9/10 years of age (late childhood).
Materials and methods: Black participants ($n=368$, 52% boys) were born into Birth to Twenty, a mixed longitudinal cohort study set in Johannesburg-Soweto from April—June 1990. Height and weight data in early (mean age = 3.6 years $\pm$ 0.6) and late childhood (mean age = 10.1 years $\pm$ 0.7) 0.7) and subscapular (SB) and tricep skinfolds in early childhood were analysed. Body mass index (BMI) values were calculated and categorised according to IOTF cut-offs as OW or normal weight (NW). Logistic regression was conducted using early childhood OW as a predictor of late childhood OW. *T*-tests were used to detect differences in early childhood BMI and skinfolds between those who did and did not track OW.
Results: An odds ratio of 6.3 (95% CI 2.9 to 13.5) was observed for a child tracking OW to OW compared with a child who was NW at early but OW by late childhood. Fourteen out of 35 OW children in early childhood tracked OW and these had greater mean BMIs and SBs of 0.8 kg/m^2 ($p=0.009$) and 1.2 mm ($p=0.012$) in early childhood respectively compared with children NW by late childhood.
Conclusion: South Africans who are OW in early childhood are at significant increased risk of being OW by late childhood. OW children most likely to track into late childhood are associated with having large BMIs and SBs in early childhood.

Disclosure: Was this work supported by industry? Yes: The Wellcome Trust (UK).
Do you act as a consultant, employee or shareholder with this industry? No.

J-18
Effects of prenatal exposures on glucose metabolism in adulthood are mediated through adiposity

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Objectives: Birth weight has been associated with the risk of type 2 diabetes in several studies. We investigated whether key prenatal influences on birth weight are associated with glucose metabolism in midlife, and the role of birth weight and adult adiposity in mediating these associations.
Methods: Data from 7799 participants of the British 1958 birth cohort attending a biomedical survey at age 45 years were analysed. Associations between prenatal exposures and HbA1c $\geq$ 6 in adulthood were examined using a series of logistic regression models. The basic model consisted of prenatal factors adjusted for sex and family history of type 2 diabetes. This was then adjusted for (i) birth weight-for-gestational age (BGA) only, (ii) concurrent adiposity only (BMI and waist circumference), (iii) BGA plus concurrent adiposity.
Results: In the basic model, SEP at birth, maternal smoking, pre-eclampsia, and pre-pregnancy BMI were independently associated with HbA1c. Adjustment for BGA had little impact on the prenatal associations, however, adjustment for adiposity attenuated the associations and odds ratios (OR) were no longer significant for all factors except for pre-eclampsia (OR = 2.03, 95% CI = 1.17—3.53).
Conclusions: Relationships between prenatal exposures and blood glucose levels in mid-adulthood are largely mediated through adult adiposity but surprisingly not through birth weight. Prenatal exposures are unlikely to affect glucose metabolism directly, but appear to act through their influence on adiposity over the life-course. The association with pre-eclampsia has not previously been reported for glucose metabolism in offspring in adulthood.

Disclosure: Was this work supported by industry? No.

J-19
The effect of maternal nutrient restriction in late gestation on plasma insulin, glucose, IGF-1 levels, and muscle fiber size and cortical bone density later in life

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Materials/methods: Twenty twin-bearing Shropshire ewes were fed either adequately (H) or a 40% restricted (L) diet

during the last 6 weeks of gestation. The ewes were sired by rams selected for either high daily growth rate (growth, G) or high cross-sectional area of *m. Longissimus dorsi* (LD) combined with minimal fat thickness above LD (meat, M). The male offspring ($n=23$) were slaughtered 5 months *post partum* and type 2 muscle fiber size in LD and *m. Biceps femoris* (BF), plasma glucose, insulin, insulin-like growth factor-1 (IGF-1) levels, femur weight, and cortical bone density were measured at slaughter.
Results: Maternal nutrient restriction during the last 6 weeks of gestation reduced birth weight (4.2 ± 0.1 vs. 3.4 ± 0.2 kg, $p=0.04$), increased type 2 muscle fiber size in BF (1337 ± 117 vs. 1727 ± 143 μm^2, $p=0.05$), reduced femur weights (169 ± 4.8 vs. 148 ± 6.0 g, $p=0.01$), and increased cortical bone density (2.3 ± 0.031 vs. 2.5 ± 0.04 g/cm^3, $p=0.009$) and tended to increase plasma glucose concentrations (4.0 ± 0.2 vs. 4.6 ± 0.2 mM, $p=0.06$). No effects were found on plasma insulin, IGF-1 concentrations nor on type 2 muscle fibre size in LD. None of the parameters measured were affected by paternal genetics.
Conclusion: The data show muscle-specific effects of maternal nutrient restriction in late gestation and suggest that both muscle fiber size and some bone parameters express compensatory growth as a result of an impaired *in utero* environment.

Disclosure: Was this work supported by industry? No.

J-20
Maternal undernutrition and a postnatal high fat diet increase GLUT2 expression in the pancreas of adult offspring concomitant with hyperinsulinemia and increased adiposity

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Background: We have previously shown that maternal undernutrition during pregnancy leads to hyperinsulinemia, hyperleptinemia, increased adiposity, and lethargy in offspring; the effects of which are amplified following exposure to a postnatal high fat diet. In the pancreas, GLUT2 plays a critical role in mediating glucose-stimulated insulin secretion. The present study investigated the impact of maternal undernutrition and postnatal high fat nutrition on GLUT2 expression in the pancreas of offspring in adult life.
Methods: Virgin Wistar rats were time mated and randomly assigned to receive food either ad-libitum (CON-group) or at 30% of ad-libitum intake (small for gestational age, SGA-group) throughout gestation. At weaning, male CON and SGA offspring were fed either a chow or high fat diet ad-libitum for the remainder of the study.
Results: At day 110, GLUT2 expression was significantly increased ($p<0.05$) in SGA offspring compared to CON offspring and was further increased in high fat fed offspring ($p=0.05$). Concomitant with elevated GLUT2 expression, plasma insulin, insulin/glucose ratio, c-peptide, leptin, and total body fat were increased in SGA offspring and further increased in offspring fed the high fat diet postnatally.
Conclusions: These data suggest that a mismatch between pre- and postnatal nutritional environments leads to a dysregulation of pancreatic function and alterations in GLUT2-mediated glucose sensing pathways resulting in hyperinsulinemia and the development of obesity in adult life.

Disclosure: Was this work supported by industry? No.

J-21
The Copenhagen Follow-up Study of Fetal Growth (COFFEG): low birth weight, but not third trimester growth restriction is associated with insulin resistance in adolescence

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Objective: To assess if third trimester growth and birth weight for gestational age have parallel effects on glucose-insulin metabolism.
Methods: Third trimester fetal growth velocity (FGV) was calculated from longitudinal ultrasound measurements, and birth weight (BW) for gestational age was assessed in a previous cohort study of 1000 high-risk pregnancies. From this birth cohort, 121 adolescents (52 male) were followed up at mean age 17.5 years (range: 16.2—19.4 years). Insulin sensitivity (SI), first phase insulin response (FPIR), and disposition index (DI) were derived from an intravenous glucose tolerance test (IVGTT) ($n=105$). Homeostasis model assessment index of insulin resistance (HOMA-IR) was assessed ($n=113$). Data were analysed in multiple regression analyses correcting for BMI and sex.
Results: BWSDS was inversely associated with basal insulin ($b=-5.8\%$/SDS, $p=0.04$)) and HOMA-IR ($b=-6.2\%$/SDS, $p=0.04$). No significant associations between BWSDS and FPIR, DI or SI were observed. The analyses showed no effect of FGV on the measured parameters.
Conclusion: In accordance with several studies, we found a negative association between BWSDS and insulin resistance, but no effect of BWSDS upon glucose-stimulated insulin secretion. To our knowledge, this is the first study to use actual measurements of fetal growth to detect an impact of intrauterine life upon later glucose-insulin metabolism. However, the lack of effect of third trimester growth suggests, that an earlier period during gestation, rather than third trimester, is the critical window of organ growth and differentiation influencing glucose-insulin metabolism later in life.

Disclosure: Was this work supported by industry? Yes: Novo Nordisk.
Do you act as a consultant, employee or shareholder with this industry? No.

J-22
Fasting plasma glucose and pregestational physical activity are main determinants of newborn macrosomia

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Objective: The proportion of macrosomic newborns is increasing. Being born large is associated with short- and long-term health risk. The purpose of the present investigation was to evaluate prospectively the contributions of modifiable maternal predictors of fetal macrosomia ($\geq$4200 g), including overweight, weight gain, plasma glucose, nutritional intake, and physical activity.
Materials/methods: Women were recruited from the booking list of the delivery unit. 553 were followed four times during pregnancy, first time 14—16 weeks. A wide range of predictive variables were subjected to univariate and multiple logistic regression analysis. Among these were: First visit body mass index (BMI) above 25, weight gain, maternal and gestational age, parity, sex, maternal subcutaneous fat (mm, subaxillary, caliper), fasting and 2 h glucose at first and third visits, and nutritional intake (energy, carbohydrate, protein, and fat in energy percent (E%)). All continuous variables were dichotomized, using upper quartile as cut point in most cases. Also physical exercise (more or less than 20 min/week) before pregnancy were studied in multiple analysis.
Results: In a model where physical exercise was excluded, apart from gestational age, parity and sex; BMI, fasting glucose at third visit and weight gain increased the risk of macrosomia (OR 3, 1.8, 1.9, and 2, respectively), whereas high protein intake reduced the risk (OR 0.5). After inclusion of physical exercise; BMI, weight gain, fasting glucose, and physical exercise (OR 1.7, 2, 1.9, and 0.4, respectively) remained independent determinants.
Conclusions: Absence of physical exercise and variables linked to "westernized lifestyle" seem to be major determinants of fetal macrosomia.

Disclosure: Was this work supported by industry? No.

K. PLACENTA

K-01
Salt overload during pregnancy alters uterine blood flow, placenta renin angiotensin system (RAS), and placenta weight

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Objective: To evaluate if salt overload during the gestation modify body weight due placenta RAS and uterine blood flow alteration.
Methods: Female rats fed normal (0.5% NaCl, NSD group) or high (8% NaCl, HSD group) salt diet since 8 weeks of age were matted at 12 weeks of age and studied at third week of gestation. Body weight (g), kidney mass, fetal (FW, g) and placenta weight (PW, g), blood pressure (mm Hg), heart rate (bpm), uterine (UBF, ml/min/g), cardiac output (CO, ml/min), and peripheral vascular resistance (mm Hg/ml/min) were measured. Plasma renin activity (PRA, ng/ml/h) and placenta angiotensin I, II and 1—7 were measured. Angiotensinogen (AGT), ACE, AT1 receptor, and TNF-α mRNA in the placenta were evaluated.
Results (mean $\pm$ EPM—$p < 0.05$, $n = 4$—6): PW was higher on HSD (0.44 ± 0.01, $n = 111$) than NSD (0.41 ± 0.01, $n = 106$). PRA was lower on HSD (5 ± 2) compared to NSD (13 ± 2). Angiotensin I was lower on HSD (227 ± 23) compared to NSD (416 ± 48) and angiotensin II (HSD: 289 ± 26, NSD: 445 ± 21) as well. CO and UBF were higher on HSD (CO: 109 ± 21, UBF: 0.7 ± 0.1) than NSD (CO: 58 ± 5, UBF: 0.4 ± 0.1). Kidney hypertrophy was observed on HSD (0.38 ± 0.02) compared to NSD (0.28 ± 0.01). AGT and TNF-α were lower and AT1 receptor mRNA was higher on HSD (AGT: 0.69 ± 0.03, AT1: 0.70 ± 0.02, TNF-α: 0.42 ± 0.03) than NSD (AGT: 0.84 ± 0.06, AT1: 0.59 ± 0.01, TNF-α: 0.51 ± 0.04). All the other evaluated parameters were not different between groups.
Conclusion: HSD during pregnancy may contribute to a higher PW through uterine hyperfusion probably by plasma and placenta RAS suppression. **Support:** FAPESP.

Disclosure: Was this work supported by industry? No.

K-02
Placental proportions affect age 7 BMI independent of birth and placental weights

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Objectives: Placental growth, an important modulator of birth weight, is variable in terms of chorionic surface area (proportional to the uterine surface available for placental supply) and disk thickness (a measure of villous arborizing growth). We hypothesize that variance in these placental proportions will account for childhood growth variance independent of birth weight.
Materials/methods: Placental data (e.g., placental weight, larger and smaller placental disk diameters, disk thickness, and umbilical cord length) collected in the National Collaborative Perinatal Project (NCPP, recruited 1959—1966) was extracted for singleton liveborns delivered at >34 gestational weeks, and restricted to the first offspring of each family ($N = 15{,}399$). Body mass index (BMI) at age 7 years was regressed against placental weight, birth weight, and estimated placental chorionic surface area (calculated from larger and smaller placental disk diameters) and disk thickness.
Results: Placental thickness and surface area are independently associated with BMI at age seven, even after adjustment for birth and placental weights. For each S.D. unit change in placental thickness, BMI at age 7 increases 0.07 units (95% CI: 0.04—0.10), per unit in placental area change, 0.06 BMI units (0.02—0.10) per unit in placental weight change it is 0.05 BMI units (0.01—0.10), and per unit in birth weight change it is 0.24 BMI units.
Conclusion: Placental proportions affect BMI independent of birth weight at age 7 years. We speculate that fetal physiology is modulated by chorionic plate area (reflecting maternal perfusion area) and extent of villous arborization (and hence the nutrient exchange surface) with potentially enduring effects.

Disclosure: Was this work supported by industry? No.

K-03

Umbilical cord length affects age 7 BMI independent of birth and placental weights

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Objectives: Umbilical cord length, through which passes ~50% of fetal cardiac output, is directly related to fetal cardiovascular resistance. We have previously shown in the National Collaborative Perinatal Project (NCPP) that umbilical cord length is related to birth weight independently of other placental growth measures. We test the hypothesis that variance in umbilical cord length (UCL) accounts for childhood growth variance independent of birth weight.
Materials/methods: Placental data (e.g., umbilical cord length, placental weight, larger and smaller disk diameters, disk thickness) collected in the National Collaborative Perinatal Project (NCPP, recruited 1959—1966) was extracted for singleton liveborns delivered at >34 gestational weeks, and restricted to the first offspring of each family (N = 15,399). Body mass index (BMI) at age 7 years was regressed against umbilical cord length, placental weight, birth weight, estimated placental chorionic surface area, and disk thickness.
Results: Umbilical cord length is independently associated with BMI at age 7, even after adjustment for birth weight and placental weight. For each SD unit change in umbilical cord length, the change in BMI at age 7 is 0.07 unit (95% CI: 0.04—0.10).
Conclusion: Umbilical cord length affects BMI independent of birth weight at age 7 years. We speculate that, given vessel length is directly proportional to vascular resistance, greater umbilical cord length results in a reduced net nutrient benefit to the fetus for each fetal—placental cardiac cycle. These data suggest that umbilical cord length modulates fetal physiology in fashion that lasts at least until age 7 years, in terms of childhood BMI.

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