Journal of Developmental Origins of Health and Disease (2009), Volume 1, Supplement 1, S1–S60

doi:10.1017/S2040174409990018

6th World Congress on Developmental Origins of Health and Disease

Oral Presentations

O-2A-1

Birth weight versus child order: which one is more important for intellectual development at 5y?

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Objective: To evaluate in 5 year-old Chilean children the relationship among birth weight, birth order and intelligence quotient (IQ) controlling the effect of maternal variables.
Methods: In a representative sample of 250 Chilean preschool children enrolled in a state child care program[1] (birth weight >2500 g), we obtained birth weight (BW) and length (BL), maternal age (MA), mother's educational level (ME) and birth order (BO) from official registries. At 5 years, children were evaluated with the Intelligence Wechsler Scale (IQ) WPPSI-R by a trained psychologist. Variables were analysed as continuous and categorical; if not normally distributed, non-parametric statistics were used; uni and multivariate analysis were done. After ethical approval by INTA's ethics committee, informed consent was obtained from parents.
Results: 49.6% were girls, average BW was 3,412 ± 454 g, (17% ⩾2500 to 3000 g, 71% >3000 to 4000 g, 12% >4000 g; BL was 49.8 ± 1.7 cm, no stunting at birth (1.6% <2 SD HAZ, WHO 2006); IQ was 92 ± 9 score, with differences by BW categories; ME (17% high, 45% middle and 36% basic); and BO (38% first, 33% second and 29% third or greater) ($p < 0.001$). Linear regression showed that these associations were stronger in adjusted models, BW explained 9% (R^2) of variability in IQ. Interaction between BW and BO was tested ($p < 0.05$).
Conclusions: This study provides evidence that the relationship between BW and IQ score is dependent on BO and that a high BW does not condition higher IQ. We confirmed that a healthy birth weight in terms of intellectual development at 5y is 3000–4000 g. Our results suggest that in low income families, the position a child holds within his/her family plus favorable nutritional status at birth, are important to maximize mental development. In countries with high prevalence of both macrosomic and low birth weight babies interventions aimed at achieving healthy weights at birth are particularly relevant.
Support: Fondecyt # 1060785 and JUNAEB.
Adjusted IQ score for all BW categories of and BO are shown in Fig 1. Firstborn children in all categories showed higher IQ scores. In children with high BW (>4000 g), the BO effect on IQ was stronger than that of BW. Several studies have shown that BO represents the social position of the child within the family, as confirmed by our results[2,3]. BW of 3000 to 4000 g showed the lowest IQ differences among categories of BO, for this BW group average IQ was closest to the expected mean normal (⩾100 IQ). Children of lower BW (<3000 g) showed no consistent relationship between IQ and BO.

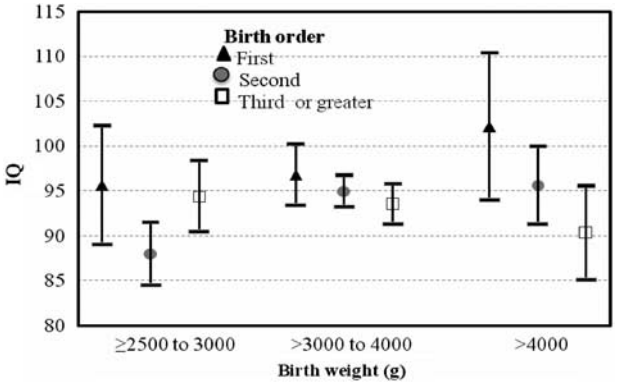

Fig. 1. Relation between birth weight and IQ score according to birth order. Mean IQ ± standard error, adjusted for mother's education level, maternal age at birth and sex of children. Reference: birth order list.

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O-2A-2

High pregnancy anxiety during mid-gestation is associated with decreased gray matter density in 6-9 year-old children

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Objective: Because the brain undergoes dramatic changes during fetal development it is vulnerable to environmental insults. There is evidence that maternal stress and anxiety during pregnancy influences birth outcome but there are no studies that have evaluated the influence of stress during human pregnancy on brain morphology.
Methods: In the current prospective longitudinal study, pregnancy anxiety was assessed serially in 35 women during pregnancy (at 19 (±0.83), 25 (±0.9) and 31 (±0.9) weeks gestation). When the offspring from the target pregnancy were between six to nine years of age, their neurodevelopmental stage was assessed by a structural MRI scan.
Results: With the application of voxel based morphometry, we found regional reductions in gray matter density in association with pregnancy anxiety after controlling for total gray matter volume, age, gestational age at birth, handedness and postpartum perceived stress. Specifically, independent of postnatal stress, pregnancy anxiety at 19 weeks gestation was associated with gray matter volume reductions in the prefrontal cortex, the premotor cortex, the medial temporal lobe, the lateral temporal cortex, the postcentral gyrus as well as the cerebellum extending to the middle occipital gyrus and the fusiform gyrus. High pregnancy anxiety at 25 and 31 weeks gestation was not significantly associated with local reductions in gray matter volume.
Conclusions: This is the first prospective study to show that a specific temporal pattern of pregnancy anxiety is related to specific changes in brain morphology. Altered gray matter volume in brain regions affected by prenatal maternal anxiety may render the developing individual more vulnerable to neurodevelopmental and psychiatric disorders as well as cognitive and intellectual impairment. This research was supported by National Institute of Health grants NS-41298, HD-51852 AND HD28413 to CAS.

O-2A-3

Cognitive functioning in individuals who were prenatally exposed to famine: results from the Dutch Famine Birth Cohort

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Objective: Low birth weight, prenatal exposure to alcohol, drugs and smoking have been associated with impaired cognitive functioning. There is evidence for effects of prenatal adversities on cognitive functioning, but studies have only been conducted in childhood and not much is known about effects later in life. We investigated whether prenatal exposure to famine was related to mid-life cognitive functioning. We explored (a) whether time of exposure did matter and (b) whether a possible relationship was mediated through poor physical health.
Methods: Data from 738 eligible members of the Dutch Famine Birth Cohort were analyzed. Cohort members were born as term singletons around the time of the Dutch famine and physically and mentally examined at age 58. 64 were exposed to famine in early gestation, 107 in mid gestation and 127 in late gestation. Control groups consisted of 231 participants born before the famine and 209 conceived after the famine. The cognitive tests administered included the AH4 test (to assess problem solving ability), immediate and delayed paragraph recall (episodic memory), a mirror tracing task (procedural memory) and a Stroop color-word incongruence task (executive functioning). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Compared to the unexposed, those exposed to famine during early gestation made on average significantly more errors on the Stroop Task (12.6%, 95% CI, 20.5 to 4.8%; $p = 0.002$). The same effect was seen after adjusting for sex, socioeconomic status (SES) and education (11.3%, 95% CI, 18.5 to 4.1%; $p = 0.002$) and after excluding inattentive and indifferent participants (10%, 95% CI, 18.2 to 1.7%; p 0.018). Only, after adjusting for sex, education and SES, had those exposed in late gestation on average significantly more errors on the Stroop task (5.9%, 95% CI, 11.2 to 0.56%, $p = 0.03$) and on the delayed recall condition of the paragraph recall task (number of errors 1.92 95% CI, 3.32 to 0.51, $p = 0.008$). None of the relationships was mediated through physical health (all p-values >0.05 for CHD, glucose intolerance, HDL, LDL and triglyceride). No effects were found on the AH4 test and mirror tracing task (all p-values >0.05 for early, mid and late exposed).
Conclusions: Adults who were prenatally exposed to famine during early gestation had significantly poorer executive functioning (Stroop). Those exposed to famine during late gestation also seemed to have poorer executive functioning and memory (Paragraph Recall). These effects were not mediated through poor health (CHD, glucose intolerance, HDL, LDL, triglyceride). The present findings are interesting as they were obtained in 58 year old individuals rather than in children or juveniles. The findings imply a long lasting effect of prenatal malnutrition on executive functioning and perhaps also memory. Future follow ups and the addition of more cognitive tests and brain imaging to the current measures will have to show whether the findings are precursors of more rapid aging due to prenatal exposure to famine.

O-2A-4

Effects of prenatal betamathasone exposure on autonomic function and cortical activity at the age of eight years – a pilot study

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

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Prenatal betamethasone (BM) treatment is used to enhance fetal lung maturation in women threaten premature labor. Recent studies have shown altered stress sensitivity in the neonate. Although the stress system interacts closely with cognitive domains, follow-up studies into child and adulthood suggest no adverse effects on neuropsychological development.

Objective: To examine, if alterations of stress sensitivity following prenatal BM exposure persist to the school age and if the are accompanied by functional anomalies of cerebral activation.

Methods: To prepare a larger study, we examined 4 male and 3 female offspring of women who received a single course of BM (2 × 8 mg 24 h apart) between 26 and 32 weeks gestation and delivered after 36 weeks gestation with normal birth weight and without need for treatment at the NICU. Children were examined at 7–8 years of age when they attended the second grade at school. Seven controls were matched for gender, age and gestational length. The children underwent the Trier Social Stress Test adapted for children. After a rest of 10 min, the child was provided with a story scenario and needed to develop the story for 5 min, followed by 5 min of mental arithmetic and 10 min of positive feedback. EEG and EKG were recorded during the entire protocol. We analyzed the EEG using linear (power spectral analysis) and nonlinear analysis. For nonlinear analysis we used an algorithm based on the Wolf-Algorithm which calculates a prediction error (PE) based on the time series in the phase space. A high prediction error stands for low causality or regularity and *vice versa*. The PE has proved to be effective in detecting distinct changes of the functional state of the brain and of acute effects of BM on complex neuronal interactions. Activity of the autonomic nervous system (ANS) was analyzed by heart rate variability (HRV) in the time and frequency domain.

Results: BM treated children showed frequency shift to higher frequencies and a higher PE than controls in the temporal EEG recordings at rest suggesting altered cortical activity ($p < 0.05$). During the story and mental arithmetic tasks, the spectral edge frequency and the PE increased in the controls reflecting cortical activation ($p < 0.05$). In contrast, the BM treated children did not show cortical activation. HRV measures did not differ between the groups at rest but BM treated children showed a higher SDNN and SDNN/RMSD quotient than controls during the story and mental arithmetic tasks ($p < 0.05$). This suggests higher sympathetic activity since the SDNN is modulated by sympathetic and vagal activity and the RMSSD only by vagal activity.

Conclusions: Prenatal exposure to a single course of BM seems to have a persisting influence on postnatal sympathetic activity accompanied by a lack of cortical activation in cognitive tasks. This effect is not tampered by preterm delivery or neonatal intensive care. The higher cortical activation at rest and the absent activation during the tasks might be an effect of a different trajectory of brain development or of the altered activity of the stress system. Considering the striking effects on the activity of the ANS and the cortex in this pilot study and the common clinical use of the therapy, a large study is warranted.

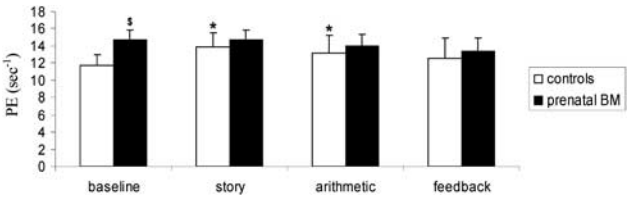

Fig. 1. Changes of the nonlinear PE of the EEG during the Trier Social Stress Test (T3 electrode). Mean + SEM; $^{\$}p < 0.05$ compared to controls, $^{*}p < 0.05$ compared to baseline.

O-2B-5

Size at birth is related to grip strength in 9 year-old Indian children: findings from the Mysore Parthenon Study

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Objective: Muscle strength is important for everyday activities and work capacity. In adults, low grip strength is a predictor of future disability, morbidity and mortality, and reduced quality of life[1–2]. Studies in adults and children have shown that low birth weight is associated with reduced hand grip strength, suggesting that intra-uterine growth and/or maternal nutrition may have permanent effects on muscle function. We analysed data from the Parthenon birth cohort study in Mysore, India to test the hypothesis that lower weight, length and mid-upper-arm circumference (MUAC) at birth are associated with reduced grip strength at age 9.5 years.

Methods: The Parthenon study is a prospective birth cohort study of 630 mothers who gave birth at the Holdsworth Memorial Hospital (HMH), Mysore, in 1997–1998, and their children, who had detailed anthropometry at birth and every 6–12 months up to the age of 9.5 years[3,4]. Hand grip strength was measured at 9 or 9.5 years in 574 children (91% of the cohort) using a Jamar dynamometer. Socio-economic status was assessed using the Standard of Living Index (SLI). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: The children were short, light and thin at birth (birth weight and birth length SD scores −1.0 and −0.4 respectively according to the WHO reference) and at 9.5

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

years (BMI and height SD scores −1.2 and −0.6). Mean grip strength was 12.7 kg (boys) and 11.0 kg (girls) (p for the difference between sexes <0.001). Birth weight was positively related to grip strength at 9.5 years; β = 0.38 kg per within-cohort SD increase in birth weight (95% CI: 0.21–0.56), p < 0.001. There were significant associations of a similar magnitude between grip strength and MUAC and length, but not skinfold thickness, at birth. Additionally, grip strength was positively related to SLI score, to 9.5-year height, BMI, and MUAC and to increases in these measurements from birth to 2 years, 2–5 years and 5–9.5 years. The associations between birth size and grip strength were attenuated, and no longer statistically significant, after adjusting for 9.5-year size.
Conclusion: Larger overall size and muscle mass at birth are associated with greater muscle strength at 9.5 years. Pre-natal muscle development may have permanent effects on muscle function, and these could be mediated partly through effects on post-natal growth. The study was funded by the Parthenon Trust, Wellcome Trust and the Medical Research Council, UK.

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O-2B-6

Increase in Weight for Age is Associated with Increased Fat-Free Mass in Healthy Children

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Objectives: Previous studies have reported a positive or U-shaped relationship between birth weight and fat-free mass (FFM) in adulthood (1-4). However, there are some contradictions between these studies and divergences in the use of techniques used to assess body composition. Therefore, the objective of this study was to determine the relationship between size at birth and body composition in childhood.
Methods: Birth weight and gestational were obtained from using maternal recall and body composition, fat mass and FFM, were assessed using dual energy X-ray absorptiometry in a cohort of children from the New York metropolitan area. Statistical methods used included Students t-test and multiple linear regression analyses. Appropriate institutional ethics committee clearance and patients consent were obtained.
Results: The mean age (±SD) of the children was 12.9 ± 2.4 years (range of 9–18 years), a mean height, weight, and BMI were 159.1 ± 13.3 cm, 54.1 ± 16.5 kg, and 21.0 ± 4.3, respectively, and a median Tanner stage of 3.0. The mean birth weight was 3.3 ± 0.52 kg with a mean gestational age of 39.0 ± 1.6 weeks. Birth weight adjusted for gestational age (AdjBwt) was negatively associated with FFM, independent of sex, Tanner stage, weight and height[2]. The statistically significant negative relationship between AdjBwt and FFM was persisted when FFM was adjusted for current body weight (adjFFM) or expressed as a percent of body weight (%FFM). To further explore this relationship, the cohort was split into the following three groups base on their change in weight for age from birth to childhood (i.e. WAZ at DXA measurement – WAZ at birth): rapid weight gain (ΔWAZ > 0.5), no weight gain ($0.5 > \Delta WAZ < -0.5$), and weight loss (ΔWAZ < −0.5). It was found that children who had positive or minimal increase in WAZ, but not those who decreased WAZ, had a negative relationship between either adjFFM or %FFM and AdjBwt.
Conclusions: Based on the results of our study, we concluded that while birth weight is negatively associated with FFM in childhood, this relationship is partly mediated by the rate of growth from birth to childhood. Support for this research was provided by the Busch Biomedical Foundation and by NIH/NIA grant number P01 AG10120.

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O-2B-7

Abdominal wall fat index in neonates: correlation with birth size

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The importance of fat distribution in early life is not well known, and few studies have investigated visceral adiposity in neonates[1].
Objective: To determine the correlation between visceral adiposity in neonates (with) and the birth size.
Methods: We measured the visceral adiposity in 118 newborns with gestational age ⩾37 weeks using the abdominal wall fat index (AWI), ratio between the maximum thickness of preperitoneal and the minimum thickness of subcutaneous fat evaluated by ultrasound[2]; an ATL HDI 5000 (CA, Bothell) with CL 4–7 MHz or CL 2–5 MHz curvilinear transducer was used. The interoperator and intraoperator reliability was assessed. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: The sonographic measurements showed good interoperator and intraoperator reliability, with intraclass

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

correlation coefficients of subcutaneous and preperitoneal fat thickness respectively: 0.96 (95% CI; 0.86 to 0.99) and 0.95 (95% CI; 0.83 to 0.99). There is a weak negative correlation between AWI and birth weight ($r = -0.197$; $p = 0.033$) but not with birth length ($r = -0.118$; $p = 0.201$), body mass index ($r = -0.138$; $p = 0.176$) and waist abdominal ($r = 0.063$; $p = 0.497$).

Conclusions: Our finding suggests that intrauterine growth restriction may contribute to visceral adiposity in early life. This could help to explain the low birth weight predisposition to accumulating visceral fat in childhood and adulthood[3,4]. We also suggest that AWI is a useful parameter for evaluating the fat distribution in newborns. However, like it was the first time that this method was used in newborns our results need to be confirmed.

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O-2B-8

High infant mortality in the past could explain current stroke mortality at Brazil?

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Objective: To verify the correlation between current stroke mortality with past infant mortality in Brazil.

Methods: In this ecological study the correlation between current stroke mortality of the 36 mains cities of Northeast and South/Southeast of Brazil and infant mortality rates of these same cities in 1950 was obtained. Coronary heart disease, stomach and lung cancer mortality rates were also calculated. We calculated sex specific mortality in 2005 for people aged 50 years or more who were thus born around 1950. The data source was the System of Information of the Ministry of Health. Kendall coefficient correlation was used to evaluate the association between infant mortality rate and adjusted mortality rates among adults in 2005.

Results: We found no correlations between infant mortality in 1950 and stroke mortality in 2005 (table). To examine the possible confounding effect of current circumstances, we also calculated correlations between adult mortality in 2005 and infant mortality in the same period. For stomach cancer, lung cancer and coronary heart disease the table also shows no correlations as well. These partial correlation coefficients indicate no association with infant mortality in 1950.

Conclusions: In Brazil stroke is the main cause of death, and this high mortality has been justified by social determinants but it was not studied yet under the developing origins of health and diseases hypothesis[1]. By this hypothesis the risk of stroke is increased by maternal influences associated with poverty[2]. We did not find a geographical correlation of stroke mortality with infant mortality at time of birth. We believe that our results could be explained by current differences in socio-economic conditions between Northeast and South/Southeast. We found that high infant mortality in the past could not explain the burden of stroke mortality in Brazil. Further studies are needed to clarify the relation between low birth weight and stroke mortality in Brazil.

Table. Relation of adult mortality (age ⩾ 50 years) in 2005 with infant mortality in 1950, controlling for region and mortality in 2005.

	Infant mortality 1950			
	Male		Female	
	S/SE **	NE*	S/SE**	NE*
Kendall correlation coefficient	tau (p value)	tau (p value)	tau (p value)	tau (p value)
All causes	−0.11 (0.525)	0.27 (0.249)	0.08 (0.631)	0.10 (0.697)
Stomach cancer	−0.13 (0.438)	−0.10 (0.699)	0.01 (0.943)	0.26 (0.268)
Lung cancer	−0.13 (0.433)	0.38 (0.098)	−0.16 (0.373)	0.15 (0.529)
Coronary heart disease	−0.08 (0.613)	0.17 (0.437)	−0.10 (0.557)	0.28 (0.218)
Stroke	−0.11 (0.508)	0.01 (0.981)	−0.14 (0.418)	0.09 (0.701)
Partial correlation coefficient				
All causes	−0.02 (0.900)	0.27 (0.223)	0.09 (0.538)	0.09 (0.677)
Stomach cancer	−0.20 (0.203)	−0.09 (0.677)	0.01 (0.950)	0.27 (0.229)
Lung cancer	−0.11 (0.457)	0.38 (0.085)	−0.16 (0.291)	0.15 (0.510)
Coronary heart disease	−0.07 (0.644)	0.18 (0.426)	−0.09 (0.542)	0.31 (0.156)
Stroke	−0.03 (0.826)	0.02 (0.941)	−0.13 (0.408)	0.10 (0.652)

Northeast*, South/Southeast**.

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O-2C-9

T-Lymphocytes from malnourished infants are characterized by being short-lived and dysfunctional cells

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Objective: It is recognised that a poor diet during pregnancy may have a long term impact on the health of the child. However, little is known about the effect of a poor maternal

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

diet on the child's immune system. There is some indication that apoptosis of immune cells in undernourished organisms might lead to an increase in susceptibility to diseases related to immune suppression. Apoptosis of lymphocytes from peripheral blood and lymphatic organs accompanying malnutrition has been poorly described, but no studies have explored the mechanisms of T cell functional perturbation in malnourished infants. Accordingly, we designed a pilot study to investigate T cell functional molecules and inflammatory cytokines in malnourished infants and to provide new immunological evidence for the clinical manifestations associated with malnutrition.

Methods: Undernourished women from a poor suburb of (Assiut), Egypt were recruited to the study upon presentation at the maternity hospital. 62 malnourished infants (28 female, 34 male) aged 15.2 ± 7.5 months (mean ± SD) and 28 healthy control infants (14 female, 14 male) aged 14.6 ± 5.4 months were studied. The phenotypic and the functional signatures of T cells were monitored using flow cytometry with different panels of antibodies. Appropriate institutional ethics committee clearance and patients consent were obtained.

Results: Malnourished infants showed significantly decreased levels of circulating IL-2 and IL-7 and increased levels of the inflammatory cytokines IL-1beta, IL-6, IL-10 and TNF-alpha. There was a significant reduction in CD3+ T cells count and an increase in apoptotic T-cells correlated with a marked up-regulation of CD95 and PD-1 expression (exhaustion marker) on $CD3^+$ T cells in malnourished compared to the control infants. Furthermore, T cells from malnourished patients expressed low levels of CD127 (IL-7 receptor alpha) and CD28 (costimulatory receptor), exhibited diminished proliferative capacity as measured by CFSE dilution assay and showed decreased cytokine production as demonstrated by intracellular cytokine staining (ICS). They also exhibited decreased AKT, STAT3 and STAT5 phosphorylation as measured by Western blot. Expression of L-selectin, CCR7 and CXCR4 receptors that mediate homing of peripheral blood lymphocytes to secondary lymphoid organs (site of immune response initiation) were markedly reduced. Decreased expression of L-selectin, CCR7 and CXCR4 was correlated with a significant reduction of T cell migratory capacity to their ligands CCL21 and CXCL12 respectively as measured by *in vitro* chemotaxis assay.

Conclusions: T lymphocytes from malnourished infants are short-lived and dysfunctional: this may increase susceptibility to major human infectious disease.

Table. Mean Fluorescence Intensity (MFI).

CXCL-12-mediated chemotaxis	MFI-CXCR4	CCl-21-mediated chemotaxis	MFI-CCR7	Apoptosis	MFI-PD1	MFI-CD95	
47.3 ± 5.9%	877 ± 6.5%	34 ± 4.6%	247 ± 5.6%	5 ± 3.5%	37 ± 3.4%	Control	
28.9 ± 4.4%	610 ± 5.8%	19.9 ± 5.3%	177 ± 5.1%	21 ± 4.3%	112 ± 4.6%	Undernourished	

O-2C-10

Immunological programming of the neonatal inflammatory response following *in utero* LPS exposure

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Objective: Preterm births constitute approximately 10% of all live deliveries in developed countries, including Australia[1]. Inflammation is a primary cause of early preterm birth[2]. Microbial invasion of the amniotic cavity, most commonly by genital tract microflora, is considered to be a leading cause of fetal inflammation[2]. Preterm very low birth-weight (VLBW) babies (>1500 g at delivery) are at an elevated risk of developing late onset sepsis and are significantly more likely to die following infection[3]. Gram negative microorganisms, notably *E. coli*, are commonly detected in late onset sepsis screening[3]. Hospitalized adults who have developed endotoxin tolerance are more susceptible to nosocomial infections and mortality[4]. Here, we investigated the potential for *in utero* developmental exposure to *E. coli* lipopolysaccharide (LPS) to program suppression of the immune response in the ovine model of fetal inflammation.

Methods: Date mated merino sheep were given intraamniotic (IA) doses of LPS or control (saline) under ultrasound guidance. Fetal cord blood was obtained 2d, 7d and 14d post initial administration following elective caesarean delivery. Monocytes were isolated for *in vitro* LPS challenge and inflammatory analysis as previously published[5,6].

Results: *In vitro* challenge with LPS induced robust expression of IL-6 (Figure 1 A + C). and H_2O_2 (Figure 1 B + D) in blood

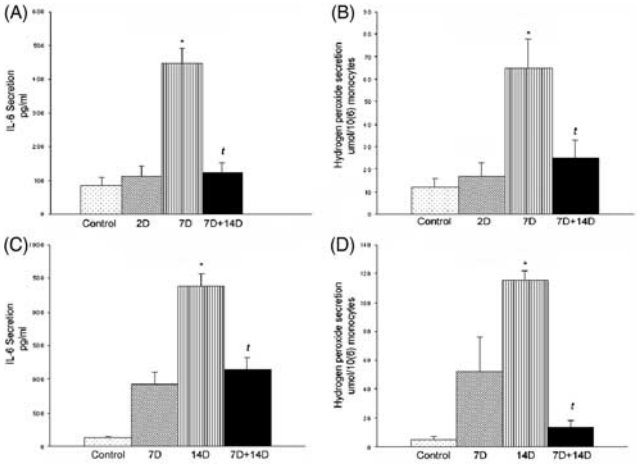

Figure 1. (A) IL-6 secretion following 2D or 7D IA LPS; (B) H_2O_2 production following 2D or 7D IA LPS; (C) IL-6 secretion following 7D or 14D IA LPS; (D) H_2O_2 production following 7D or 14D IA LPS; *$p < 0.05$ v control; $^t p < 0.05$ v single IA LPS dose at 7D or 14D.

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

monocytes exposed to IA LPS at 7d or 14d prior to delivery. In contrast, stimulation of monocytes exposed to repeated administrations of IA LPS (at 2D + 7D or 7D + 14D) prior to delivery exhibited a blunted production of IL-6 and H_2O_2. ***Conclusions:*** *In vitro* administration of LPS to blood monocytes previously stimulated once at either 7D or 14D prior to delivery induced robust expression of IL-6 and H_2O_2, indicating robust immune activation. Repeated IA administration resulted in a blunted response to LPS, with significant reductions in both IL-6 and H_2O_2 expression. This data provides evidence that repeated *in utero* exposure to LPS in the ovine model of fetal inflammation may program the down-regulation of subsequent inflammatory responses to gram negative microorganisms and as such may contribute to late onset sepsis in VLBW preterm infants.

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O-2C-11

Severely preterm birth protects from atopy – Helsinki Study of Very Low Birth Weight Adults

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Objective: Preterm birth has been related to a reduced risk of atopy[1–4]. However, not all studies have been consistent[5,6]. The aim of this study was to evaluate atopic predisposition in young adults born prematurely at very low birth weight (VLBW).

Methods: The study comprised 166 VLBW adults (birth weight < 1500 g) and 172 term-born controls (birth weight >2500 g), age range 18 to 27 years. Atopic predisposition was assessed by skin prick tests (SPT) for six common aeroallergens (birch, timothy, mugwort, dog, cat, dermatophagoides pteronyssinus), and by a symptom questionnaire (asthma, allergic rhinitis, and atopic eczema). Multivariate logistic regression analyses were adjusted for the following confounding factors: birth weight being small for gestational age (SGA, <−2SD); mode of delivery; maternal smoking during pregnancy; sex; current height, body mass index, age, weekly smoking; paternal and maternal atopy; parental education level; having cats or dogs at home currently or during the first year; number of elder siblings, and occurrence of bronchopulmonary dysplasia. Appropriate institutional ethics committee approval and participants' informed consent were obtained.

Results: VLBW subjects had a lower risk of having at least one positive SPT reaction than their term born counterparts (adjusted OR 0.43, CI 0.23–0.79, p = 0.007). Number of positive SPT reactions increased with increasing gestational age as well as with increasing birth weight (Figure). Cumulative incidences of atopic symptoms did not differ between VLBW subjects and controls, but in a subgroup of extremely low birth weight (ELBW, birth weight <1000 g) premature subjects, the risk of atopic eczema was lower than in controls born at term (adjusted OR 0.26, 95% CI 0.07–0.90, p = 0.033).

Conclusions: Young adults born prematurely with VLBW have reduced rates of atopic predisposition. The rates are lowest in those born smallest or most immature. The study has been supported by the Foundation for Pediatric Research, Finland.

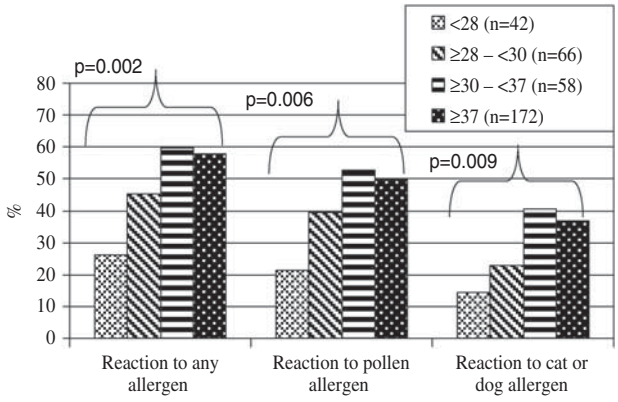

Fig 1. Percentages of subjects having positive skin prick test reactions according to gestational age at birth (p for trend between the groups calculated by χ^2 test).

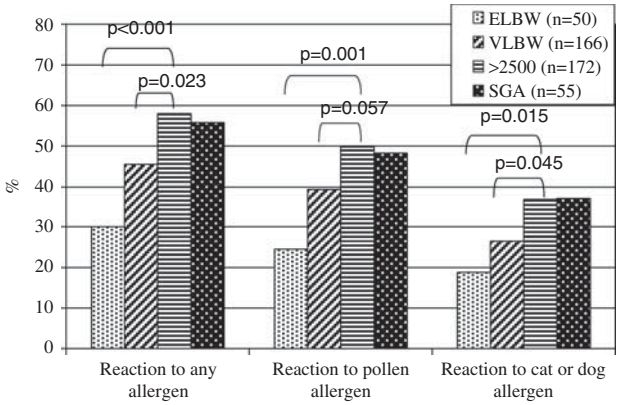

Fig 2. Percentages of subjects having positive skin prick test reactions according to birth weight (p for difference between pointed groups calculated by χ^2 test).

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

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O-2C-12

Effect of supplemental folic acid in pregnancy on childhood asthma: a prospective birth cohort study

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Objectives: Recent studies in mice[1] and infants[2] indicate that supplemental folic acid intake during pregnancy adversely affects respiratory health. To our knowledge, no previous study has reported the simultaneous effect of the *timing, dose and source* of maternal folic acid (synthetic supplement form) and folate (natural dietary form) during pregnancy on childhood asthma. We aimed to investigate these effects on childhood asthma at 3.5 and 5.5 yrs.

Methods: We analysed data from a prospective birth cohort study (n = 557) in Australia. A representative sample of mothers was recruited from 4 antenatal clinics in early pregnancy from 1998 to 2000. At 3.5 yrs 490 and at 5.5 yrs 423 mothers and children (88% and 76% of the original sample) participated in the study and did not have missing data for the main outcome and confounder variables. The primary outcomes were doctor-diagnosed asthma, obtained by maternal-completed questionnaire when the child was 3.5 and 5.5 yrs. Maternal folate intake from diet and supplements was assessed by food frequency questionnaire administered in early (<16 wks) and late (30–34 wks) pregnancy. Potential confounders considered were: maternal education; maternal smoking; maternal intake of Vitamin E, Vitamin A, and Zinc, birth characteristics; breastfeeding duration; and maternal asthma. Institutional ethics clearance and patients consent were obtained.

Results: Folic acid taken in supplement form in late pregnancy was associated with an increased risk of asthma at 3.5 yrs (RR 1.26, 95% CI 1.08–1.43) which was robust to adjustment for carefully measured confounders. The association was comparable at 5.5 yrs but did not reach significance (RR 1.17, 95% CI 0.96–1.42) in univariable models. Neither supplemental intake in early pregnancy nor dietary folate was associated with asthma in off-spring at either age in univariable analyses. However, there were significant interactions for asthma at 5.5 yrs between cigarette smoking and dietary folate in early pregnancy (RR for interaction term 1.73, P = 0.01) and between parity and folic acid in late pregnancy (RR for interaction term 2.18, P = 0.03).

Conclusions: Our findings indicate that supplemental folic acid in late but not early pregnancy is associated with childhood asthma. Our study is consistent with, and effectively translates to humans, a previous study in mice demonstrating folate-impaired methylation in pregnancy results in alteration of the fetal immune phenotype and adverse respiratory health outcomes[1]. The timing of the effect of folic acid supplementation seen in our study may give insight into the biological pathway involved, particularly as an effect after the first trimester coincides with the development of the fetal immune system. Following confirmatory research, current recommendations about folate intake in pregnancy should consider the timing of folate administration to maximise the neuro-protective effects of folic acid whilst minimising potential adverse postnatal respiratory effects. This study was supported by grants from the Faculty of Health Sciences at the University of Adelaide, the Dairy Research and Development Corporation, the Channel 7 Children's Research Foundation and the NHMRC (Grants 349548, 465455, 465437).

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O-3A-13

Twin conception reduces birth weight and gestation length in sheep, regardless of fetal number in late gestation

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Objective: Twins are commonly born both early and small and these factors are associated with adverse long-term health outcomes. The dogma is that constraints of uterine size and placental supply in late gestation are responsible, but there are no data to support this. The periconceptional period is known to be important for fetal development and gestation length. We hypothesised that conception *per se* as a twin would alter fetal growth and the timing of birth.

Methods: Time-mated twin-bearing ewes were randomly assigned to fetal reduction of one twin (reductions; $n = 32$) or a sham procedure (twins; $n = 20$). Singleton-bearing ewes (singles; $n = 23$) also underwent a sham procedure. Fetal reduction was achieved through ultrasound-guided transabdominal

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

injection of 1.5 mL 2 M KCl into the heart or chest cavity on day 42 of gestation (term = 147 d). Gestation lengths, birth weight, and other anthropometric measurements were recorded at, or shortly after, birth. Data were compared by ANOVA with post-hoc correction and are presented as mean (SEM).

Results: Gestation length was shorter in twins than singles (147.0 (0.32) vs 148.3 (0.34) d; $P < 0.05$); reductions had intermediate gestation length (147.3 (0.46) d). Birth weight was greatest in singles, intermediate in reductions, and least in twins, each group being statistically different (singles 6.60 (0.16) Kg; reductions 5.82 (0.14) Kg; twins (5.23 (0.12) Kg; all comparisons $P < 0.0001$). Other anthropometric data were similar between reductions and twins, with singles significantly larger than these two groups ($P < 0.05$). These findings are not altered by adjustment for gestation length.

Conclusion: Following reduction of twin pregnancies to singletons in early gestation, birthweight and gestation length remain less than in singletons. These are the first experimental data demonstrating that gestation length and birthweight in twin fetuses are determined, at least in part, in early gestation. However, the significantly greater birthweight in reductions compared with twins indicates that there is additional constraint of growth in late gestation. Birth weight and gestation length are therefore influenced by fetal number both at the time of conception and in late gestation. Further studies will determine whether this altered growth has long-term implications for growth and risk of disease.

O-3A-14

Does feeding regimen between pregnancies affect the birth weight and survival of lambs?

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Objective: Maternal nutrition during the peri-conceptional period and pregnancy has important effects on fetal growth trajectories[1,2,3].Yet, little is known about the effect of feeding or liveweight gain of the dam between pregnancies and prior to the beginning of the peri-conceptional period on growth of the offspring. Our objective was to determine whether liveweight change from the weaning of the previous set of lambs through to 71 days before rebreeding (a period of 42 days) affected birth weight and survival of lambs to weaning at 60 days.

Methods: 1367 ewes were randomly allocated to either 'High' (liveweight gain 89 ± 3 g/day; n = 693; initial live weight 70.7 ± 0.3 kg) or 'Maintenance' (liveweight gain 9 ± 3 g/day; n = 674; initial live weight 70.6 ± 0.3 kg) nutrition from 113 to 71 days prior to joining. Ewes were then all managed as one group until weaning. Lambing and weaning data were collected from 868 ewes and their lambs. Lambs were weighed within 12 hours of birth and at an average age of 60 days. The study was conducted under large-scale, pastoral farming conditions in New Zealand. Massey University Animal Ethics Committee approval was obtained.

Results: Nutritional treatment of the ewe did not affect any of the outcomes measured, however, initial liveweight of the ewe was positively related to pregnancy status, number of lambs born per ewe, total birth weight and total weaning weight of the litter. Age of ewe (range 3 to 9 years) was inversely related to number of lambs reared per ewe, survival of a litter, and total weaning weight of litter, and positively related to mean birth weight of litter.

	High treatment			Maintenance treatment			Probability value	
	n ewes	LSM	SE	n ewes	LSM	SE	Treatment	Initial liveweight
Number of fetuses detected (0–3)	690	1.90	0.03	674	1.92	0.03	0.615	<0.0001
Number of lambs born per ewe	431	1.90	0.03	437	1.89	0.03	0.772	0.014
Number of lambs weaned per ewe	431	1.54	0.03	437	1.55	0.03	0.750	0.282
Survival of lambs per ewe (%)	431	82	1	437	84	1	0.287	0.861
Total birth weight of litter (kg)	431	8.1	0.1	437	8.1	0.1	0.785	0.0008
Mean birth weight of litter (kg)	431	4.35	0.04	437	4.41	0.04	0.264	0.136
Total weaning weight of litter (kg)	431	38.2	0.8	437	37.9	0.8	0.827	0.001

LSM = least squares mean, SE = standard error.

Conclusions: Nutrition from weaning of the previous set of lambs until 71 days prior to rebreeding did not affect the productivity of ewes in the following pregnancy and lactation; however, live weight of the ewe at the previous weaning was positively related to pregnancy status, number of lambs born, and total birth and weaning weight of the litter. This indicates that aspects of maternal nutritional status or body composition at the time of weaning the previous set of lambs, rather than subsequent feeding regimen, can influence the weight of lambs from the next pregnancy.

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O-3A-15

Does dam parity affect the lambing performance of two-year-old ewes and their lambs growth and survival to weaning?

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https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

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Objective: This study was undertaken to investigate if the performance of two-year-old ewes and their lambs to weaning was affected by the parity of their dam, or the birth rank of the two-year-old ewe itself. It might be expected that those born to 2nd parity dams would display higher performance levels. Previously we have shown that at 8 months of age, young ewes whose dam had failed to conceive at 8–9 months of age were less likely to achieve puberty than those born to dams which had conceived at 8–9 months of age or which were not presented for breeding[1]. There were however no differences in the liveweight of these young ewes from their birth to 18 months of age[1,2]. Those ewes born as singletons to either primiparous or 2nd-parity dams were heavier to one year of age than those born as a twin but not thereafter[1].
Methods: This study utilises 2-year-old ewes who were born to 2-year-old dams which had either been; successfully bred at 8–9 months of age and the re-bred at 19 months (therefore were of 2nd parity, (2ndpar), n = 68), failed to bred at 8–9 months of age but were successfully bred at 19 months (therefore first parity, (1stpar-2ndbreeding), n = 50) or were not presented for breeding at 8–9 months and then successfully bred for the first time at 19 months (therefore first parity, (1stpar), n = 44). The two-year-old ewes also consisted of those that were singleton- or twin-born (n = 69 and 93 respectively). The two-year-old ewes were weighed in late pregnancy prior to the lambing period. Lambs (n = 231) born to the two-year-old ewes were weighed within 12 hours of birth and at average day 88 of lactation (L88). At the lambs first weight the ewes mothering behaviour was recorded[3]. Massey University ethics committee clearance was obtained.
Results: Dam parity did not influence the liveweight of the two-year-old ewes in late pregnancy ($P > 0.05$, 63.4 ± 0.8, 64.0 ± 0.9 vs. 63.7 ± 0.9 kg for 2ndpar, 1stpar-2ndbreeding and 1stpar two-year-old ewes respectively) or L88 ($P > 0.05$) nor did it affect mothering ability ($P > 0.05$). The birthrank of the two-year-old ewes did not influence their liveweight in late pregnancy ($P > 0.05$) or their mothering ability ($P > 0.05$). Although at L88, those two-year-old ewes, which were singleton-born were heavier ($P < 0.05$) than those twin born (61.4 ± 1.0 vs. 59.1 ± 0.8 kg). The lamb's birth weight was not affect by its grand-dams parity ($P > 0.05$, 5.8 ± 0.1, 5.9 ± 0.1 vs. 5.9 ± 0.1 kg for those whose grand-dam was 2ndpar, 1stpar-2ndbreeding and 1stpar respectively) nor it's dam birthrank ($P > 0.05$, 5.9 ± 0.1 vs. 5.9 ± 0.1 kg for those born to single- or twin-born two-year-old ewes). Similarly, lamb weight and survival at L88 were not affected ($P > 0.05$) by grand-dam parity or dam birthrank.
Conclusion: Overall the data indicates there were no grand-dam parity or dam birthrank affects on the lamb to weaning or parity effects on the liveweight of the ewe. Therefore these potential affects need not be considered when selecting replacement animals although, previously it has been shown that dam parity may affect attainment of puberty[1]. Single-born ewes were found to be heavier at the weaning of their lambs, an affect reported previously to two years of age[4]; this has potential to affect their future productive performance.

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O-3A-16

Are there inter-generational effects of grand-dam size and dam nutrition during pregnancy on lamb performance to 1-year-of age?

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Objective: This study was undertaken to investigate the potential inter-generational effects of grand-dam size and nutrition during pregnancy; along with the intra-generational effects of dam birthrank, on performance traits of male and female lambs with the aim of providing advice to farmers regarding the selection of replacement stock.
Methods: The grand dams of the animals in the present study were either 'Large' (60.8 ± 0.18 kg) or 'Small' (42.5 ± 0.17 kg) and offered either maintenance or *ad-lib* levels of nutrition on herbage only from day 21 of pregnancy until day 140[1]. The maintenance feeding regimen was designed to ensure that total ewe liveweight increased with expected conceptus mass. We have previously shown intra-generational effects of dam nutrition on liveweight at birth and in early life and on lactational performance of female offspring at 2-years-of-age[1,2,3]. This study investigates potential inter-generational effects on the offspring born to these 2-year-old ewes. Post-weaning ewe and ram lambs were managed under commercial farming conditions. Live weights of ram lambs (n = 92) from birth to 6-months of age were recorded as were carcass characteristics at slaughter. Live weights of ewe lambs (n = 114) from birth to 1 year of age were recorded. The proportion of ewe lambs reaching puberty at 8 months of age was recorded. Appropriate institutional ethics committee clearance was obtained.
Results: Liveweight, growth rate and carcass composition of male lambs were not ($P > 0.05$) affected in an inter-generational manner by grand-dam size or pregnancy nutrition, or intra-generationally by dam birthrank. Live-weight and growth rate of female lambs were not ($P > 0.05$) consistently affected in an inter-generational manner by grand-dam size or pregnancy nutrition. Lambs with small grand-dams were less likely ($P < 0.05$) to attain puberty

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

compared to those with large grand-dams (59.9% vs. 83.6%). Grand-dam nutrition (72.0 vs. 74.8% for ewe lambs whose grand dams were offered either maintenance or *ad-lib* levels) and dam birthrank (77.9 vs 68.3% for ewe lambs whose mother was single or twin born) did not ($P > 0.05$) influence the attainment of puberty.

Conclusion: Overall, the data suggest that there are few inter-generational effects of grand-dam size or pregnancy nutrition on performance traits, aside from the effect of grand-dam size on the attainment of puberty in female lambs. The data also suggest that there are no intra-generational effects of dam birthrank, in terms of the performance traits measured. Therefore, inter-generational effects of grand-dam size and pregnancy nutrition, and intra-generational effects of dam birthrank need not be considered in the selection of offspring. However given that this is one of the first inter-generational study in sheep, further investigations are required before firm conclusions can be made.

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O-3B-17

High fat diet during critical windows of development alters adrenal cortical and medullary enzyme expression in adult male offspring

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Objective: Previously, we reported that rats fed a maternal high fat (HF) diet throughout life and during pregnancy and lactation resulted in offspring with increased adiposity and hyperleptinemia in adulthood[1]. Since leptin has been shown to have an inhibitory effect on adrenal steroidogenesis and to increase epinephrine synthesis, which increases satiety, we hypothesized that key enzymes in the adrenal cortex and medulla involved in steroidogenesis and catecholamine synthesis would be altered in these offspring.

Methods: Virgin Wistar rats were assigned to one of three experimental groups: 1) control dams fed standard chow diet throughout dam's life and pregnancy and lactation (CON), 2) maternal high fat dams fed high fat diet from weaning and throughout pregnancy and lactation (MHF), and 3) dams fed standard chow diet throughout life until conception, followed by high fat diet throughout pregnancy and lactation (PLHF). At weaning, all offspring were fed either chow (C) or high fat (HF) diet for the remainder of the study, resulting in 6 experimental groups. Offspring were killed at 160 days, adrenal tissue dissected and either frozen for mRNA analysis or fixed for immunohistochemistry. Data are presented for male offspring and were analyzed by ANOVA with Tukey's post-hoc test for multiple comparisons (significance $p < 0.05$).

Results: PLHF-HF, MHF-C and CON-HF offspring demonstrated increased adrenal adrenocorticotrophic hormone receptor (ACTH-R) mRNA levels compared to controls (CON-C) ($p = 0.003$). Steroidogenic acute regulatory protein (StAR) mRNA levels were unaffected by maternal di*et al*one, but when in combination with a postnatal HF diet, MHF-HF offspring demonstrated increased StAR mRNA expression compared to CON-C and to PLHF-HF offspring ($p = 0.01$). Medullary phenylethanolamine N-methyltransferase (PNMT) mRNA expression was increased in MHF-HF compared to MHF-C offspring and in CON-HF compared to MHF-C offspring ($p = 0.008$). Leptin receptor (ObRb) was localized primarily to the adrenal medulla and may suggest an underlying mechanism regulating PNMT expression levels.

Conclusions: These studies suggest that a HF diet or a mismatch between the pre- and postnatal diets leads to changes in key enzymes that regulate adrenal steroidogenesis and catecholamine synthesis. These changes may impact downstream corticosterone production in response to stress and may impact appetite regulation. In our model, the effects of leptin on adrenal function appear to be directed towards increasing medullary catecholamine synthesis rather than inhibiting steroidogenic capacity. We speculate these offspring may have increased adrenal sensitivity to ACTH, as evidenced by increased ACTH-R and StAR expression. These findings suggest that the regulation of sympathoadrenal function, in addition to the regulation of adrenal steroidogenesis, is influenced by maternal and postnatal HF nutrition.

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O-3B-18

Does lower birth order amplify the association between high socio-economic status and central adiposity in young adult Filipino males? Evidence supporting the Mismatch Hypothesis

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Objective: To test the hypothesis that lower birth order, a known determinant of reduced fetal growth and subsequent birth size, amplifies the positive association between socio-economic status and central adiposity in young adult males from a lower-income, developing country context.

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

Methods: The Cebu Longitudinal Health and Nutrition Survey is an ongoing community-based, observational study of a one year birth cohort (1983). The analyzed sub-sample includes 970 young adult males, mean age 21.5 y (2005) with no missing data on variables of interest. Multivariable linear regression models were used to test for associations between birth order and continuously measured waist circumference (WC), or the log odds of central adiposity (waist circumference >85 cm). Covariates included maternal factors hypothesized to influence later offspring central adiposity (height, arm fat area, age, and smoking behavior during pregnancy) and socioeconomic status (SES) in young adulthood. Interactions among independent variables were tested using the appropriate product terms. We also explored body mass index (BMI) growth curves for individuals cross-classified by firstborn status (versus *not*) and young adult socio-economic status (*high* versus *low*). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Lower birth order was associated with higher WC and increased odds of central adiposity, even after adjustment for SES in young adulthood and maternal characteristics that could impact later offspring adiposity. For example, an increase in birth order was associated with 17% reduction in the odds of central adiposity (OR 0.73, 95% CI 0.57 to 0.93). Furthermore, a strong, positive association between SES and WC, or central adiposity, was amplified in individuals characterized by lower birth order (Figure 1). Firstborn status was associated with rapid early-postnatal increases in BMI. This was amplified in high SES firstborns, who also had the highest BMIs in young adulthood (Figure 2).

Conclusions: This evidence supports the mismatch hypothesis, which posits that maternal constraint of fetal growth acts to program developing physiology in a manner that increases susceptibility to the obesogenic effects of modern environments.

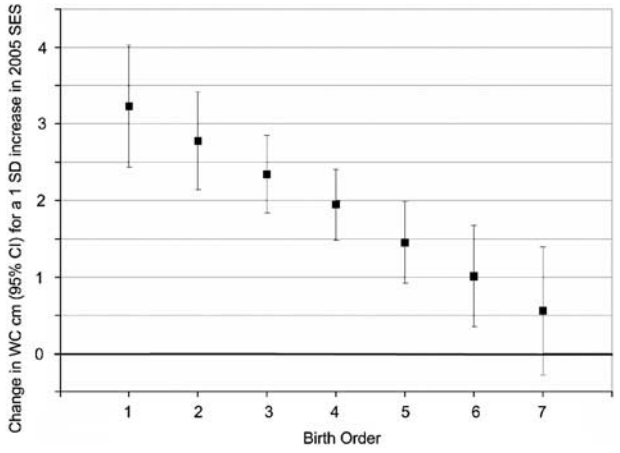

Figure 1. The impact of young adult socioeconomic status on waist circumference (cm) is amplified for lower birth orders in a sample of 970 young adult Filipino males.

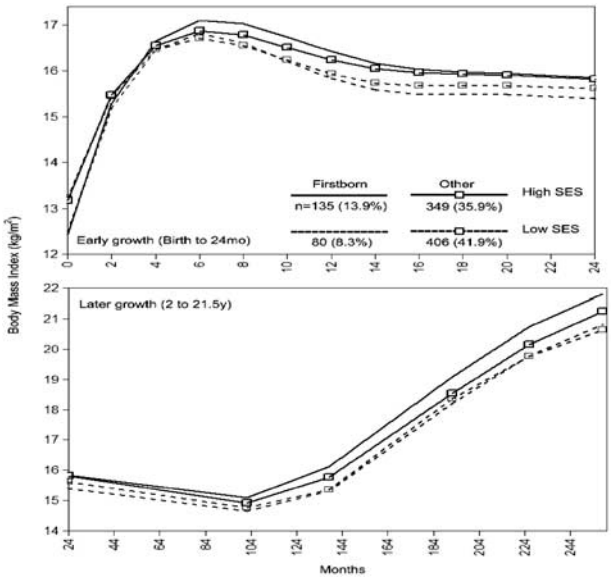

Figure 2. Body mass index growth curves for groups of males defined by firstborn status and socioeconomic status at birth.

O-3B-19

Gene-environment interactions define the relationship between the fat mass and obesity-associated gene, fetal growth and childhood obesity

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Objective: The fat mass and obesity-associated (FTO) gene variants have been consistently associated with childhood and adult obesity across multiple populations and racial groups; however, the influence of polymorphisms in FTO on fetal and early life growth is unknown. This study examines the influence of the rs9939609 variant in the FTO gene on fetal and early life growth and investigates the gene environment and gender interactions.

Methods: Participants in the Raine Birth Cohort study were recruited at 16-18 weeks gestation. Analyses focused on 1,079 singleton-birth Caucasians with complete data in antenatal life and childhood. Associations between the rs9939609

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

variant, fetal growth trajectories and anthropometric measures at birth and throughout childhood were assessed. Analyses were repeated in 3,512 singleton-birth Dutch Caucasians (Generation R Cohort). Appropriate institutional ethics committee clearance and participants' informed consent were obtained for both cohorts.

Results: The allele frequency for obesity risk allele (A) of the rs9939609 variant was 0.39 in both the Raine and Generation R cohorts. In non-smoking mothers, the rs9939609 AA genotype was associated with intrauterine growth restriction; however, this effect was reversed in mothers who smoked during pregnancy. This effect, although small, accumulates over time and is consistently modified by maternal smoking for head circumference (HC) (Interaction $P = 0.007$), abdominal circumference (AC) (Interaction $P = 0.007$), femur length (FL) (Interaction $P = 0.02$), and estimated fetal weight (EFW) (Interaction $P = 0.001$). No association was detected between HC/AC ratio and the rs9939609 AA genotype indicating symmetrical growth restriction. The pattern of modification by maternal smoking of the association between the rs9939609 AA genotype and birth anthropometrics was consistent across birth-weight (BW) ($P = 0.01$) and birth length ($P = 0.04$); a similar trend was observed in all neonatal day 2 anthropometry including skinfold thicknesses. Postnatally, the AA genotype was associated with increased BMI by age 14 years, although the effect size was greater in males (Interaction $P = 0.0001$). No interaction between AA genotype and maternal smoking was observed for postnatal growth characteristics. Consistent effect sizes and directions from birth to the 1st year of life were replicated in the Generation R Cohort.

Conclusion: FTO influences fetal growth trajectories in the 3rd trimester and early life growth. Maternal smoking during pregnancy consistently modified (reversed) the effect of FTO on fetal growth. By early adolescence, FTO is associated with increased BMI as shown in previous studies, and this effect was greater in males. This study provides evidence of gene-environment interactions influencing the relationship between fetal growth and adult obesity and raises the prospect of early life interventions. *These authors contributed equally to this work.

O-3B-20

Dysregulated adipogenic transcription factor (PPARγ) activity leads to programmed obesity in IUGR offspring

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Objective: Enhanced adipogenesis is one of key features of programmed obesity. Adipogenesis occurs via induction of adipogenic transcription factors, in particular PPARγ, which promote both adipocyte differentiation and lipid storage. We have established a rat model of maternal undernutrition that results in IUGR newborns which paradoxically develop adult obesity. As IUGR offspring exhibit upregulation of the PPARγ prior to the development of obesity, we sought to investigate the mechanism for the paradoxically increased PPARγ. PPARγ transcriptional activity is regulated by co-repressors and co-activators. Sirtuin (SIRT1) is a stress-response and chromatin-silencing factor that represses the activity of PPARγ by recruiting corepressors (nuclear receptor corepressor, NCoR and silencing mediator for retinoid and thyroid hormone receptor, SMRT). This complex binds to specific DNA sequences in the promoter region of PPARγ target genes, and inhibits their transcription. Conversely, SRC1/TIF2 co-activators directly activate PPARγ. We thus hypothesized that alteration in corepressors and/or coactivators contributes to dysregulated PPARγ activity in IUGR adipocytes, leading to enhanced lipid accumulation and programmed obesity.

Methods: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to 21. All pups were nursed by Control dams and weaned at 3 weeks to ad libitum feed. At 1 day and 9 months of age, adipose tissue was analyzed for protein expression (Western Blot) of co-regulators in IUGR and Control male offspring. Data are normalized to γ-actin and presented as fold change.

Results: IUGR offspring show marked differences in expression of PPARγ co-regulators. Corepressors: At 1 day of age, IUGR pups showed changes consistent with starvation with significantly increased protein expression of SIRT1 (1.5-fold, $P < 0.01$) and SMRT (2.6-fold, $P < 0.001$) as compared to Controls. However, the expression of NCoR was significantly reduced (0.5-fold, $P < 0.01$) in IUGR pups. In contrast, at 9 months of age IUGR adults, now obese, had decreased expression of SIRT1 (0.5-fold, $P < 0.01$) and SMRT (0.2-fold, $P < 0.01$) with unchanged NCoR levels. Coactivators: At 1 day of age, IUGR pups had increased protein expression of SRC1 (2.5-fold, $P < 0.001$) though the expression of TIF2 was comparable to Controls. Conversely, at 9 months of age showed increased expression of both, SRC1 (5.5-fold, $P < 0.001$) and TIF2 (2-fold, $P < 0.001$).

Conclusions: Consistent with their growth restricted status at 1 day of age, the adipose protein expression of PPARγ corepressors (SIRT1 and SMRT) is increased, though the expression of coactivator (TIF2) is unchanged. In contrast and consistent with obesity, NCoR expression is down-regulated whereas that of SRC1 is upregulated in 1 day old IUGR pups. Thus, dysregulation of NCoR and SRC1 at an early age in IUGR newborns may impact PPARγ modulation and hence its activity. Furthermore following obesity, upregulation of coactivators enhances PPARγ-mediated lipid accumulation and programmed obesity. These findings strongly suggest that NCoR and/or SRC1 are the primary PPARγ regulators contributing to programmed obesity in IUGR offspring.

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

O-3C-21

Estimating dairy intake in the distant past using focus group methodology: relevance for radiation dose estimation from nuclear testing in Kazakhstan

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Objective: In 1998, the US National Cancer Institute conducted a study with 2994 subjects exposed during childhood to radioactive fallout from nuclear weapons tests conducted in Kazakhstan between 1949 and 1962. Exposure to external radiation was well-documented but data about internal dose, mainly from consumption of dairy foods, was limited. Due to the long time since exposure, a well developed strategy was necessary to obtain the needed information and to encourage accurate memory recall. We required rreliable information tailored specifically to the dose reconstruction algorithm. These data will be used to investigate the relation of internal dose received during childhood to appearance of thyroid cancer precursor lesions in adulthood.

Methods: Because many study subjects were too young at the time of the nuclear tests to recall their dietary consumption and existing sources of archival data were limited, it was necessary to interview parents and other village residents who cared for children during this time. Focus group data were collection in Kazakhstan in 2007, which involved three 8-person focus groups of women in each of four exposed settlements. Women (mean age = 75.3 years) were asked to recall age-specific consumption information for their children in the 1950's. Data were collected on intakes of milk and other dairy products from cows, goats, horses, and sheep. These data were then used to estimate internal dose for individuals in the 1998 study since their exact age at the time of exposure was known.

Results: Women gathered in each village for a 2-hour facilitated discussion of lifestyle and dietary habits during the time of the nuclear testing. Information obtained from the focus groups were used to derive the ethnicity-, gender- and age-specific probability distributions on individual consumption rates of milk and dairy products. Children of both Russian and Kazakh ethnicity typically consumed cows' milk with limited consumption, by Kazakhs only, of mares, goats' and sheep milk and fermented mares' milk. Both groups ate dairy products, such as sour milk and soft cottage cheese, but with different patterns of consumption. These data, combined with physical dosimetry data related to fallout, are used to generate stochastic individual dose estimates to be used in dose-response analyses. The relative efficiency of internal compared with external radiation exposure will be evaluated.

Conclusion: This study demonstrated the utility of the focus group methodology to obtain quantitative data for dietary intakes in the distant past.

O-3C-22

Birth weight – breast cancer revisited – is the association confounded by familial factors?

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Objective: To investigate whether the association between birth weight and risk of breast cancer could be confounded by familial factors, such as shared environment and common genes.

Methods: Eligible were all female like-sexed twins of the Swedish Twin Registry, born 1926–1958 and alive in 1973. Data was obtained from birth records, and the final study population with reliable birth weight data was 11,923 twins. Hazard ratios (HR) for breast cancer according to birth weight were estimated through Cox regression, using robust standard errors to account for the dependence within twin pairs. Paired analysis was performed to account for potential confounding by familial factors. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: In the cohort analysis, high birth weight (≥3,000 g as compared to 2,500–2,999 g) increased the risk of breast cancer diagnosed at or before the age of 50 years (adjusted HR 1.57 [95% CI 1.03–2.42]) but not with breast cancer with a later onset (adjusted HR 0.80 [95% CI 0.57–1.12]). Being born light (<2,500 grams) was not statistically significantly associated with breast cancer risk compared to the reference. From 2,500 grams and upward, a 500 gram increase in birth weight conferred a HR of 1.62 (95% CI 1.16–2.27) for breast cancer diagnosed at or before 50 years. This risk remained in analysis within twin pairs (HR 1.57, [95% CI 1.00–2.48]).

Conclusion: In the present study, findings indicate that the association between birth weight and breast cancer risk, only seen in women diagnosed early (≤50 years), is not confounded by familial factors. This finding indicates that the prenatal experience is of importance for breast cancer risk in younger ages. In the continued efforts to understand how fetal factors influence breast cancer etiology, our findings

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

support further investigation of prenatal exposures or events not related to familial factors. Support: Swedish Cancer Society (4594-B01-01XAC, 4594-B04-04XAB), Swedish Council for Working Life and Social Research (2004-0174, 2004-1654, 2007-0231), Swedish Research Council (K2006-71X-14676-04-2, K2008-54X-20638-01-3), European Union funded Network of Excellence Lifespan (FP6 036894).

O-3C-23

Quantifying apoptosis by flow cytometric method in mouse embryos exposed to valproic acid

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Cell proliferation, differentiation and apoptosis play a major role in morphogenesis and organogenesis of the developing embryo. Previous studies suggest that teratogens may cause alteration in apoptotic patterns in the developing embryo leading to fetal malformations. Few studies have quantified apoptosis in early teratogen exposed embryos. Valproic acid (VA) is commonly used to treat seizures and psychiatric disorders. Administration of VA during first trimester of pregnancy causes NTDs. Nonspecific stimulation of the mother's immune system has been shown to reduce various teratogen induced fetal malformations including neural tube defects in rodents.

Objective: In this study, apoptotic changes in embryos from valproic acid (VA) exposed dams were quantified using a protocol that was developed in our lab using flow cytometry.

Methods: Apoptotic levels were quantified in head regions of VA exposed gestation day 9 embryos. Embryonic cells were disassociated enzymatically followed by differential staining with Annexin-V and propidium iodide to differentiate apoptotic, dead and live cells.

Results: Apoptosis levels increased in the head regions of VA exposed embryos ($18.1 \pm 2.2\%$) compared to controls ($12.7 \pm 1.9\%$). We measured changes in apoptosis levels in embryos from the non specific immune stimulated dams exposed to VA. Non specific immune stimulation using IFN γ reduced VA induced apoptotic levels in the head regions of the embryos. TUNEL assay, used to localize apoptotic regions, also showed increased apoptosis along the open neural folds of the VA exposed embryos compared to controls.

Conclusions: This is the first report using flow cytometry to evaluate decreased apoptosis in early teratogen exposed embryos by nonspecific maternal immune stimulation. NIH grant K01RR16241-01e.

O-3C-24

Programming of increased early mammary tumour risk is additively and independently augmented by post-pubertal obesity

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It is well established that individuals of low birth weight are at increased risk of developing insulin resistance, type-2 diabetes and cardiovascular disease. Low birth weight and high birth weight both been associate with increased risk of breast cancer[1]. The mechanistic bases of these relationships are not known. However, studies in humans and animal models suggest that the early environment, and in particular early nutrition, plays an important role. The detrimental effects of low birth weight on type 2 diabetes risk are exaggerated by adult obesity. The interaction between birth weight and adult obesity on breast cancer risk is unknown, however a recent study showed that women of lower birth weights exposed to excess weight during adulthood had higher levels of free 17beta-estradiol over an entire menstrual cycle compared with women with high birth weights and adult overweight[2].

Objectives: The aim of this study was therefore to investigate the combined effects of low birth weight and adult obesity on mammary tumor risk using our established rat model of poor early growth.

Methods: Wistar rat dams ($n = 24$ per group) were fed either a control diet (containing 20% protein) or an isocaloric low-protein (LP) diet (containing 8% protein) throughout pregnancy and lactation. Control and LP offspring were then administered 50 mg/kg nitrosomethylurea NMU to induce mammary tumours as described previously[3]. All animals were weaned onto standard laboratory chow. After the onset of puberty (7 weeks of age), half of the females from each litter were randomised to receive either standard laboratory chow (C and LP groups) or a highly palatable obesity inducing diet (C-HPD and LP-HPD groups). A separate cohort of animals of all four groups (4 months of age) was used to investigate the effects of obesity on candidate mRNA and protein expression without carcinogen interference.

Results: Early-mammary tumor incidence was doubled in LP offspring ($p < 0.05$). Diet-induced obesity increased risk in both C-HPD and LP-HPD groups ($p < 0.05$) such that the highest incidence was observed in LP-HPD group. This increased incidence paralleled an upregulation of insulin

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

receptor ($p < 0.05$) and progesterone receptor ($p < 0.05$) in mammary glands, as well as increased plasma insulin and leptin concentrations.

Conclusions: Early nutrition has an important impact on mammary gland development and influences future susceptibility to tumor risk. This risk is further enhanced by diet-induced weight gain. Increased insulin and progesterone signaling may provide an underlying molecular mechanism and potential targets for intervention.

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O-4A-25

Early life exposures and adult mortality in Puerto Rico

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Objective: Childhood conditions have been shown to have important effects on the health of older adult Puerto Ricans[1]. More recent research has further demonstrated that season of birth, an indicator of early life exposure to poor nutrition and infectious diseases, has strong effects on heart disease and diabetes among older adult Puerto Ricans[2,3]. In this paper, we examine the effects of season of birth on adult mortality in this same population.

Methods: Using both waves of the PREHCO (Puerto Rican Elderly: Health Conditions) study ($n = 4291$, wave one 2002–03 and wave two 2006–07) we calculate the overall mortality rate and mortality rates for different subgroups. Those born in rural and urban settings and younger (60–74) and older (75+) age groups are of particular interest because they may have been exposed to different mortality risks. Using logistic regression we then estimate the effects of early life exposures (season of birth, infant mortality at birth, childhood health, childhood SES, low knee height, mother's risk of exposure to malaria) on adult mortality, controlling for age, gender, obesity, respondent's educational level and adult behavior (smoking and exercise). We also estimate separate models for rural/urban and younger/older age subgroups. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Higher mortality rates for those born in the third quarter (July–September), the peak season for infectious diseases and the beginning of the lean season when unemployment was high and nutritional supplies waning. The effects of these seasonal exposures at birth on adult mortality are strong even after controlling for other early life and adult factors. This is particularly evident for older adults (ages 75+) and for those born in urban settings where infectious disease loads during the first year of life were consistently high. The results are in contrast with those found for heart disease and diabetes[2] where strong seasonal effects were noted for adults 60–74 years old born in the countryside and during the fourth quarter (October–December), towards the end of the lean season.

Conclusions: This study suggests that those born when infectious disease loads are high are more susceptible to dying from the influences of these early life exposures in older adult life. The contrast of these results with earlier results[2] is partially explained because of differences in early life exposures between younger and older cohorts. Older cohorts (75+) were exposed to higher infectious disease loads at birth affecting adult mortality but not heart disease and diabetes. Younger cohorts (60–74 years) were born during significant mortality decline confounding the manifestation of seasonality effects on adult mortality but permitting their manifestation on heart disease and diabetes. Support source: This research was supported by National Institute on Aging Grant K25 AG027239. Research work for University of Wisconsin–Madison researchers are also supported by NIA core grants (R24 HD47873) and (P30 AG017266).

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O-4A-26

Socio-economic position over the lifecourse and all cause mortality at age 50–87 years: results from a Swedish birth cohort

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Objective: The relationship over the life course between socioeconomic position (SEP) and mortality is of ongoing interest on many levels, particularly in terms of policy development. Research has demonstrated that both childhood and adult SEP predict adult mortality risk. From the life-course perspective different models have been proposed for how SEP may affect health. Two models which have received particular attention are the accumulation model, and critical (or sensitive) period models[1]. We test whether accumulation or critical period models provide the best fit to data from a large Swedish cohort, adapting approach presented by Mishra and Nitsch *et al*[2].

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

Methods: Our study population is the Uppsala Birth Cohort Multigenerational Study (UBCoS Multigen)[3], which consists of males and females born between 1915–1929 in Uppsala, Sweden. SEP across the life course was measured at three time points: at birth; in adulthood (age 30–45); and in later life (age 50–65). We restricted the present analyses to the 2589 men and 2049 women who were still alive in 1980, at which point they were aged 50–65, and with information on SEP (manual or non-manual) at all 3 time points. Our outcome was whether or not the participants were still alive on 31st December 2002. To test whether the accumulation or the critical period models provided the best fit to the data, we compared a series of nested models (representing either the accumulation or critical period models) with a fully saturated model.[2] Logistic regression was used to model the relationship between SEP and all cause mortality from 1980–2002. To adjust for possible cohort or period effects, birth year was categorised into three bands (1915–19, 1920–24, 1925–29) and included in all models. The analyses were stratified by sex. Appropriate institutional ethics committee clearance was obtained.

Results: Our study found a greater difference in all-cause mortality risk at age 50–87 between adult manual and non-manual social class for both men and women. In women, the model which best fit the data was one with critical periods in adulthood and later life. Women of non-manual SEP, either in adulthood or in later life were at a lower risk of mortality compared with those who of manual SEP (adulthood: odds ratio (95% confidence intervals): 0.80 (0.66, 0.99); later life: 0.81 (0.66, 0.99). For men, the model with a critical period in later life provided the best fit, with those of non-manual SEP having a lower risk of mortality (0.66 (0.56, 0.78)) compared with men in manual occupation.

Conclusions: Our results support the notion that interventions during adult life can lower all-cause mortality risk at age 50–87 years in the Swedish population. Further research is being undertaken with respect to SEP during childhood at age 10. Funding: Swedish Research Council and Swedish Council for Working Life and Social Research.

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O-4A-27

Resveratrol reduces lipid peroxidation and increases sirtuin1 expression in adult animals programmed by neonatal protein restriction

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Objective: Protein restriction (PR) during lactation programs for lower body weight[1] and it has been reported that energy restriction is associated with higher longevity in rodents, which may be related to a lower oxidative stress[2]. Resveratrol is a natural phytoalexin found in grapes and has been associated with protective effects against oxidative stress. Additionaly, it is the main natural activator of sirtuin1 (SIRT1), a key molecule involved in aging process and lipid peroxidation[3]. Here, we evaluated the oxidative stress and the effect of resveratrol (RSV) over lipid peroxidation and hepatic expression of SIRT1 in adult animals from protein restricted mothers during lactation.

Methods: Lactating Wistar rats and their pups were divided into a control (C) group, fed a normal diet (23% protein), and a protein-restricted (PR) group, fed a diet containing 8% protein. After weaning (21days), pups in both groups were fed a normal diet until they were 180 days old. At the 160th day, the animals were separated in four groups: control, control + RSV, PR and PR + RSV. RSV was given (30 mg/kg/day by gavage) for 20 days. Lipid peroxidation was measured by thiobarbituric acid reactive substances (TBARs). Total antioxidant capacity (TAC) was evaluated by measuring the scavenging activity of the plasma on the 2, 20-diphenylpicrylhydrazyl (DPPH) and SIRT1 expression in the liver was evaluated by *Western blotting*.

Results: PR animals showed a higher TAC (+34%, $p < 0.05$) without changing in lipid peroxidation and the expression of SIRT1. The treatment with RSV increased TAC (+22%, $p < 0.05$) only in the control group without effect on lipid peroxidation and SIRT1 expression. PR animals treated with RSV had lower lipid peroxidation (−53%, $p < 0.05$) and higher SIRT1 expression (+17%, $p < 0.05$) without any further increase in TAC.

Conclusions: Maternal protein restriction during lactation programs the offspring for a higher antioxidant capacity. These animals are more sensitive to treatment with RSV, which results in lower lipid peroxidation and higher SIRT1 expression. These protective effect can be attributed to RSV reducing ROS production in mitochondria and indirectly by the activation of SIRT1, which regulate important cell functions, such as increase resistance to oxidative stress. Support: FAPERJ and CNPQ.

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O-4A-28

Prenatal glucocorticoid exposure increases brain infarction after stroke in the aged rat

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https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

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Ample of evidence suggests an increased risk of low birth weight babies for cardiovascular diseases in later life. Low birth weight may be the result of poor nutrition supply and/or increased levels of stress hormones that inhibit proliferation. In agreement with, prenatal glucocorticoid exposure induces hypertension in the young adult rat.[1] Previously we have shown that prenatal dexamethasone (DEX) exposure at the dose used clinically to enhance fetal lung maturation increases the renal and the autoregulated cerebral vascular tone in the aged rat by endothelium-dependent and independent mechanisms.[2] This may affect redistribution of cerebral blood flow after stroke.

Objective: We hypothesized that prenatal glucocorticoid exposure leads to larger brain infarctions after stroke. We examined this effect at older age because the incidence of stroke increases with age.

Methods: Pregnant dams received saline or 170 μg/kg DEX at day E19 and 20 equivalent to 2×12 mg DEX administered to a 70 kg pregnant woman. At 1.75 years of age, stroke was induced in male and female offspring ($n = 8$ in each group) by reversible middle cerebral artery occlusion (MCAO) for 30 min using the suture model. Six additional animals were sham-operated. Motor outcome was assessed using the ladder rung walking test one and four weeks after MCAO. Volume of the infarct core was quantified morphometrically using cresyl violet staining and that of the infarct core plus surrounding neurons with functional disturbances using MAP2. Reactive gliosis was determined using GFAP. Due to its anatomy, ischemic lesions in the hippocampus were quantified separately.

Results: Brain infarctions included the hippocampus, basal ganglia, subcortical regions and parts of the frontoparietal cortex. While the regions showing reactive gliosis were only slightly larger than the infarct core ($p < 0.05$ or in tendency), the tissue volume of MAP2 loss was six to ten times larger ($p < 0.05$) independent of sex and treatment. Prenatally DEX treated males ($p < 0.05$) but not females suffered from larger infarct cores than controls (Fig. 1). Both, DEX males and females also showed larger volumes containing neurons with functional disturbances (MAP2, $p < 0.05$, Fig. 1). Reactive gliosis was increased in tendency in DEX males and females. In the CA1 to CA4 region of the hippocampus, DEX males and females also showed larger necroses and areas containing functionally altered neurons ($p < 0.05$). In agreement with the larger brain infarctions, motor outcome was worse in the DEX treated males and females one week after MCAO ($p < 0.05$). In contrast to controls, these animals showed poor signs of functional recovery four weeks after MCAO ($p < 0.05$).

Conclusions: Prenatal DEX treatment induces larger brain infarctions and poor motor outcome after stroke possibly due to insufficient compensatory cerebral vasodilatation. The effect and its mechanisms require further attention because of the relatively high number of women that are clinically treated with the dose of DEX used.

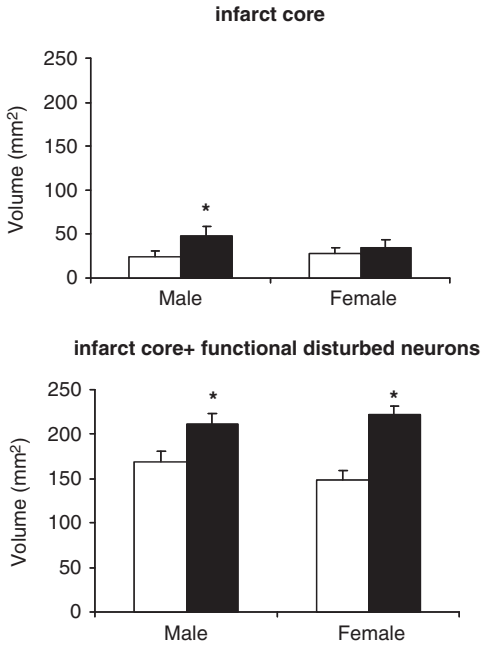

Fig. 1. Volume of brain infarctions (left) and infarctions plus surrounding functionally altered neurons (right). Controls – white, DEX – black; mean + SEM; *$p < 0.05$ compared to controls.

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O-4B-29

Relation of early weight gain with body size, body composition and fat distribution at the age of 5 in a prospective birth cohort: the ABCD study

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Objective: We assessed the relation of prenatal and postnatal weight gain with body size, body composition and fat distribution in children at age 5. We hypothesized that prenatal weight gain (represented by standardised birth weight) as well as accelerated postnatal weight gain are each independent determinants of body size (BMI), body composition ((fat mass (FM), fat free mass (FFM)) and fat distribution (waist-height-ratio (WHtR)) at the age of 5. In addition, we expected that standardised birth weight and subsequent accelerated weight gain exert a synergistic effect.

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

Methods: Prospective community-based birth cohort study (ABCD study) with repeated anthropometric measures during the first 5 years of life. Accelerated postnatal weight gain was assessed in 3 periods: immediate (first 6mo), infant (6mo-14mo) and childhood (14mo-5 yrs). At age 5, weight, height, FM, FFM (bioelectrical impedance analysis) and WHtR were determined. The association between standardised birth weight and accelerated postnatal weight gain (3 periods) with BMI, FM, FFM and WHtR was assessed. Adjustments were made for all concomitant growth measures. Furthermore, interactions of standardised birth weight with all 3 postnatal growth measures were examined, in addition to interactions between gender and all 4 determinants. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: In preliminary analyses, 717 children were included. Standardised birth weight and accelerated weight gain during the immediate, infant and childhood period were each positively associated with BMI, FM, and FFM. WHtR was only associated with immediate and childhood accelerated weight gain. The association between childhood accelerated weight gain and FM was more pronounced in girls compared to boys. No significant interactions between birth weight and accelerated postnatal weight gain in the 3 time periods were found.
Conclusions: Increased birth weight and accelerated postnatal weight gain (immediate, infant and childhood) were each independent determinants of larger body size, fat and fat free mass in 5 year old children. A more central fat distribution was associated with immediate and childhood accelerated weight gain. Furthermore, no synergistic effect of accelerated postnatal weight gain was demonstrated in the relation between birth weight and the studied outcome variables. Consequently, accelerated weight gain during each of the 3 time-periods in the first 5 years of life may be an important modifiable factor for the prevention of juvenile obesity. Financial support was granted by ZonMw.

	BMI (ß, 95%CI)	FM (ß, 95%CI) §	FFM (ß, 95%CI)§	WHtR (ß, 95%CI) ¶
BW (SDS)	0.45 (0.34–0.56)‡	0.28 (0.14–0.42)‡	0.34 (0.19–0.48)‡	0.20 (−0.05–0.44)
Immediate AWG (ΔSDS 1–6 mo)	0.39 (0.28–0.50)‡	0.19 (0.05–0.33)‡	0.35 (0.20–0.49)‡	0.29 (0.03–0.55)*
Infant AWG (ΔSDS 6–14 mo)	0.48 (0.32–0.64)‡	0.45 (0.25–0.65)‡	0.23 (0.02–0.43)*	0.01 (−0.36–0.38)
Childhood AWG (ΔSDS 14 mo–5 yrs)	0.84 (0.70–0.98)‡	♂ 0.37 (0.14–0.61)† ♀ 0.80 (0.52–1.07)‡	0.52 (0.33–0.71)‡	0.68 (0.35–1.01)‡

AWG: accelerated weight gain, § additionally adjusted for height at age 5, ¶ WHtR was multiplied by 100. *p < 0.05, †p < 0.01, ‡p < 0.001.

O-4B-30

Newborn size, postnatal growth and cardiovascular risk factors and body composition at 12 years (Pune Maternal Nutrition Study)

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Objective: To study associations of size at birth and post natal growth with cardiovascular (CV) risk factors and body composition at the age of 12 years in Pune Maternal Nutrition Study (PMNS).
Methods: PMNS was set up to study the association between maternal nutrition and fetal growth in a rural Indian population. 753 live deliveries took place. The offspring are followed up longitudinally with repeat anthropometry every 6 months (weight, height, head circumference, mid-upper arm circumference-MUAC and skinfolds), with a follow up rate of more than 90%. Cardiovascular risk factors (blood pressure, lipids, fasting glucose, insulin resistance by HOMA) and body composition (DXA) were assessed at 12y. We used principal components analysis (PCA) to summarize the phenotypic measurements at birth. The first component had high positive loadings for all birth measurements, indicating that this was a measure of overall size. The second component had strong positive loadings for skinfolds and negative loadings for length, head circumference and MUAC. We used this component as an indicator of the 'thin-fat' newborn phenotype. Growth refers to change in size (any anthropometric measurements) over time. We used Royston's method to calculate conditional SD scores for growth[1] and used them in regression models, along with size at birth, to determine associations of faster or slower growth at different time points with outcomes at 12y. Appropriate institutional ethics committee clearance and parent and child consent were obtained.
Results: Compared with an international references (NCHS) children were light (weight SD −1.4), short (height SD −0.80) and thin (BMI SD −1.30) at 12y. Larger overall size at birth (principal component 1) and 'thin-fat' phenotype (component 2) at birth did not predict CV risk factors at 12y. Rapid gain in weight, height and skinfolds from 9y onwards predicted higher CV risk factors at 12y ($p < 0.01$ for all). Unlike in many western studies in adults, early growth from birth to 6 months was not related to 12y CV outcomes. There were positive associations, of approximately equal strength, between the 'thin-fat' birth phenotype (component 2) and both fat and lean mass at 12y (standardized $\beta = 0.11$, $p < 0.001$ for fat mass and $\beta = 0.12$, $p < 0.001$ for lean mass). Faster growth in weight and skinfolds from 6y onwards was associated with higher fat mass. Faster growth in height (at every age from birth to 12y) and MUAC (from 6y onwards) was associated with higher lean mass.
Conclusions: Our results suggest that body composition is programmed in utero, while phenotype at birth was not related to CV risk factors at 12 years in this rural Indian population. Conventional CV risk factors are significantly related to postnatal growth. Therefore primordial and primary prevention of cardiovascular disease would have to

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

start in intrauterine life and continue through childhood. Support: The Wellcome Trust, UK.

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O-4B-31

Growth patterns in early childhood and final attained stature: data from five birth cohorts from low and middle-income countries

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Growth failure is cumulative, and short stature is associated with multiple indices of reduced human capital. Few studies have been able to address in a single analysis specificity of timing of growth failure and comparison across populations at varying planes of nutrition.

Methods: We analyzed data from birth cohorts in Brazil, Guatemala, India, Philippines and South Africa (n = 4659). We used data on length at birth, 12 mo, 24 mo and mid childhood to construct cohort- and sex- specific conditional length measures. We modeled adult height as a function of conditional length in childhood. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: The 5 cohorts experienced varying degrees of growth failure. As adults, the Brazil sample was 0.35 ± 0.89 standard deviations (SD) below the reference, while adult Guatemalans were 1.91 ± 0.87 SD below the reference. All 5 cohorts experienced a nadir in height for age Z-score at 24 mo. Birth length and growth failure in the period from birth through 12 mo were the most strongly associated with adult height. Growth in the periods 12–24 mo and 24 mo to mid-childhood showed inconsistent patterns across tertiles of adult height.

Conclusions: Despite variation in the magnitude of cumulative growth failure across cohorts, the 5 cohorts show highly consistent age-specific associations with adult stature. Growth failure prior to age 12 mo was most strongly associated with adult stature. These consistencies speak to the importance of interventions to address intrauterine growth failure and growth failure in the first 12 mo of life.

O-4B-32

Life-course BMI trajectories and adult cardiovascular disease risk factors

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Objective: High BMI is associated with increased risk of cardiovascular disease (CVD). Prevalence of obesity continues to increase across all age groups, with younger generations now experiencing the obesity epidemic at earlier life stages than older generations. As a consequence, child-to-adult BMI trajectories have changed over the generations, yet few studies examine associations between life-course BMI trajectories and adult CVD risk. A simple approach of conditional linear models is often used to examine the influence of BMI increase between two ages on later outcomes. But the extent to which BMI trajectories are associated with adult CVD risk factors is not well characterized due to methodological challenges posed by repeated BMI measurements at different developmental stages and outcomes in later life. We aim to investigate the influence of BMI and rate of BMI growth at different life stages on adult systolic blood pressure (SBP) and HDL-cholesterol (HDL-C) in a nationwide cohort followed from birth to 45y.

Methods: Data are from the 1958 British birth cohort, all born in one week in March 1958. Approximately 17,000 live-births have been followed-up to mid-adulthood. Outcomes are SBP (n ≈ 9,300) and HDL-C (n ≈ 8,000) measured at age 45y. Body mass index (BMI, height(kg)/weight(m)^2) was derived at ages 7, 11, 16, 23, 33, and 45y. BMI measures at all ages, SBP and HDL-C at 45y were all treated as response variables. We used a spline model with random coefficients for the repeated BMI measures allowing for distinct slopes for childhood and adulthood trajectories. The spline model was modelled jointly with response variables SBP and HDL-C. Correlations between BMI at age 7, child and adult BMI slopes, and adult (45y) BMI with SBP and HDL-C were estimated from the joint model. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Higher BMI at 45y, but not at 7y, was associated with higher SBP and lower HDL-C. BMI (45y) was positively correlated with SBP (r = 0.25 for males; 0.28 for females), and negatively correlated with HDL-C (−0.31 and −0.41 respectively). Rate of BMI increase was associated with SBP and HDL-C: for BMI slope in childhood, there was a positive correlation with SBP (0.21 for males; 0.17 for

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

females) and a negative correlation with HDL-C (−0.13 and −0.32 respectively). Associations were stronger for adult slope of BMI growth (0.27 and 0.34 respectively for SBP; −0.44 in both sexes for HDL-C).
Conclusions: Although pre-pubertal BMI was not associated with SBP and HDL-C in mid-life, large BMI gains in childhood and adulthood, which led to higher adult BMI, were both associated with an adverse CVD risk profile, as indicated by SBP and HDL-C at age 45y. These findings suggest that control of excessive weight gains at any life stage and prevention of adult obesity will have a beneficial effect on adult risk factors for CVD.

O-4C-33

Clinical and gonadal hormones characteristic during puberty in girls born small for gestational age or appropriate for gestational age

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There are limited and controversial data concerning pubertal characteristics in girls born small for gestational age (SGA).
Objective: The aim of the study was to characterize pubertal development longitudinally in matched healthy girls born either SGA or appropriate for gestational age (AGA) recruited from the community.
Methods: Inclusion criteria were breast Tanner Stage II and a normal body mass index (BMI). Girls were followed for three years with a complete physical exam, bone age (Greulich and Pyle) estimates, pelvic ultrasound and measurement of gonadal hormones. Appropriate institutional ethics committee clearance and informed consent were obtained.
Results: Twenty five AGA and 15 SGA girls completed the study with similar bone ages (12,6 vs 13,1 years(y)), delta bone age (3.1 vs 3.2 y), and delta height (19 ± 3 vs 17 ± 4 cm) in AGA vs SGA respectively (p = >0.05 l). Height (−0.09 ± 0.2 vs −0.98 ± 0.28) was significantly lower in SGA (p < 0/05), but BMI was not (p = 0.19). Body composition was similar in both groups (abdominal circumference, waist/hip and fat percentage) in the beginning and follow up. Sixty percent of the total group achieved breast Tanner 5 and 38% Tanner 4 at the third year. SGA girls advanced a little faster during the first two years. In the third year 52% of SGA vs 50% of AGA had Tanner 4 of pubic hair and 32% SGA vs 44% AGA were in Tanner 5 (p = >0.05). Ferriman score and ovarian volume and number of follicles between AGA and SGA were not significantly different nor was age of menarche (corrected by maternal age of menarche) 12 ± 0.2y (AGA) vs 12.6 ± 0.2y (SGA). BMI in the third year was inversely correlated to age of menarche in the whole group (r = −0.45 p < 0.01). A greater delta between weight at entry to the study and birth, was correlated with a lower age of menarche only in the SGAs (r-0.58 p = 0.01). In the third year basal and post-stimulated (LHRH-a) levels of gonadotropins and androgens (17OH progesterone, testosterone, adrostenediondione, DHEAS) were similar in both groups and did not show differences related to weight or catch up growth. Estradiol was higher in the AGA group (97.1 ± 10.8 vs 67.7 ± 16.6 pg/ml, p = 0.016) and it was directly correlated with higher delta BMI (BMI in third year minus at entry) only in the SGAs r = 0.89 p < 0.01.
Conclusion: Unselected SGA girls show a slightly faster pubertal development but no differences in external genitalia and gonadal hormonal patterns. Girls who experienced a higher weight catch up had an earlier age at menarche and higher levels of basal estradiol. Support: Fondecyt 1030610.

O-4C-34

Maternal protein restriction: fetal and ovarian development in adult rat offspring

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Maternal nutrient restriction impairs overall fetal development with long-term outcomes, including infertility in the adult offspring. The gestational food restriction may act as a stress factor that induces abnormal developmental/differentiation of ovarian cells in later life. The activation of several intracellular pathways in this disorder could affect follicular cell survival and proliferation in adulthood. Follicular development and atresia seem to depend upon a sophisticated balance between survival and atretogenic molecules. AT2 receptor (AT2R) present in *atretic* follicles, granulosa cells and follicle-stimulating hormone receptors (FSHR) is the most important follicular survival factor. Heat shock proteins (HSPs) also act as molecular chaperones, allowing cells to adapt to graded environment changes and to survive in adverse conditions.
Objective: To investigate the effects of protein restriction *in utero* on the initiation of pubertal and sexual development of female offspring by expression and localization of key proteins that regulate follicular development in the ovaries.
Methods: Rats were fed either a normal protein diet (NP) or a low protein diet (LP) during pregnancy. Pup weight and

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

ano-genital distance was recorded at birth. Onset of puberty was defined as the time of vaginal opening. Sixteen-wk old rats were anaesthetized and their ovaries collected, weighed, fixed and processed in paraffin, cut to 5μm and processed for immunocytochemistry for marking and visualization of HSP90 and 70, FSHR, PCNA and AT2R, or immediately frozen in liquid nitrogen and processed for Western blot quantification of protein.

Results: We found a significant reduction in LP birth weight (NP, 6.05 ± 0.13 vs LP, 5.66 ± 0.09; $p = 0.02$) and reduced ano-genital distance (NP, 0.87 ± 0.02 vs LP, 0.76 ± 0.03; $p = 0.006$). A significant reduction of ovarian area [NP, median = 8; minimum = 2.5 and maximum = 11 values) vs LP, median = 5.3; minimum = 3 and maximum = 7.8 values; $p = 0.01$] and density of primordial/primary follicles [NP, median = 1.7; minimum = 0.3 and maximum = 4.4 vs LP, median = 1.3; minimum = 1 and maximum = 2.8 number/mm^2; $p = 0.04$] was verified by morphometry. Secondary follicle density was unaffected [NP, median = 0.9; minimum = 0.2 and maximum = 1.3 vs LP, median = 0.9; minimum = 0.3 and maximum = 2.5 number/mm^2; $p = 0.4$]. The LP pubertal onset was significantly delayed [NP, median = 38; minimum = 34 and maximum = 42 vs LP, median = 37; minimum = 32 and maximum = 46 days; $p = 0.02$]. An increased PCNA [NP, 4144.26 ± 124 vs NP, 4481.8 ± 254; $P < 0.05$] as well as HSP90 expression in LP offspring [NP, 737.17 ± 176.43 vs LP, 1005.14 ± 96.89; $P < 0.03$] was observed using Western Blot,. Immunocytochemical labeling to HSP90 and PCNA confirmed these data preferentially in the follicular cells where the nucleus is positive to PCNA. Immunocytochemical study of ovaries showed an increased HSP70 expression accompanied by fall in FSHR and AT2R immunostained positive cells.

Conclusion: The current study demonstrates in LP group a decreased FSHR expression in parallel with a reduction in ovarian area and follicular density. The fall in FSH receptor may compromise follicular survival, otherwise the reduction of ovarian AT2R expression could reflect the small number of atretkic follicle. The present findings suggest that an adaptive anti-apoptotic effect of HSP90 and 70 might directly or indirectly stimulate follicular cell proliferation rescuing ovarian function in adult offspring from maternal protein-restricted pregnancy. Support: FAPESP.

O-4C-35

Sex differences in infant growth and body composition: implications for the developmental origins of adult reproductive function

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Objective: Birth weight and ponderal index at birth have been linked to the timing of sexual maturation and adult reproductive function, and researchers have hypothesized that these associations reflect alterations in the hormonal axis due to nutritional stress encountered during fetal development. The relationship between sex hormones and early postnatal growth has received less attention despite documented sexual dimorphism in infant size, and the known activation of the hypothalamic-gonadal axis at this time. This study aimed to identify if sexual dimorphism in growth, body composition, and endocrine levels were evident during infancy.

Methods: The data used to address these objectives come from a longitudinal sample of 32 American infants, 15 boys and 17 girls, measured weekly during the first 15 months of life. Length, weight, body and limb circumferences, and trunk and limb skinfold thickness were collected following standard techniques. Estradiol and testosterone were assessed from fecal samples using methanol extraction and assayed using microassay RIA techniques. Sex differences in growth and body composition measures were assessed using Mann-Whitney two-sample rank sum non-parametric tests. Repeated-measures mixed models were used to assess the association between sex steroid levels and growth and body composition measures, controlling for sex, age and potential confounders. Repeated-measures logistic models were used to assess the effect of hormone levels on weekly incremental gains in length, weight, and body composition measures. These models were stratified by sex and age to account for non-linear developmental trends. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Male infants had higher birth weight and length than female infants and remained heavier and longer over the first year of life. Sexual dimorphism was documented in adiposity and adipose tissue distribution, with female infants having higher ponderal indices, greater skinfold thickness, and a more central distribution of adipose tissue from 4 months of age through the end of the first year. These sex differences in body composition were associated with hormonal differences. Higher estradiol levels were associated with greater subscapular and thigh skinfolds, and reduced abdominal and thigh circumference in male infants in the first 6 months of life. In girls, higher estradiol predicted greater suprailiac skinfolds and larger abdominal and arm circumferences. Testosterone showed no significant associations with skinfold measures in either sex during this period, but was associated with narrower hips in boys and increased upper arm circumference in girls.

Conclusions: These results point to, already in infancy, sexually dimorphic body morphologies that are known to reflect sex-specific hormonal profiles in adolescents and adults. Such evidence highlights the possibility that sex steroid production in human infants could prime growth and reproductive maturation in response to energy availability, providing a mechanism underlying the developmental origins

of adult reproductive function. Support: The Emory University Seed Fund, the Robert H. Woodruff Fund and the National Science Foundation Dissertation Improvement Grant (BCS#0424307).

O-4C-36

Advanced pubertal timing in subjects born preterm-Helsinki Study of Very Low Birth Weight Adults

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Objective: We have reported that young adults with very low birth weight (VLBW) are more insulin resistant and glucose intolerant as well as shorter than those born at term[1]. Risk of diabetes in adults and short final height are associated with early pubertal development[2,3]. Early maturation has been observed in subjects born small for gestational age (SGA)[4,5]. We explored the timing of pubertal development in subjects born preterm.

Methods: The study comprised 154 premature VLBW subjects (birth weight <1500 g), and 200 controls born at term[1]. Of the VLBW subjects 52 were born SGA and 13 had neurologic impairment (e.g. cerebral palsy). We used growth data collected from archived child welfare clinic and school healthcare records to estimate the age at onset (take-off) and peak height velocity of pubertal growth spurt, and the age at attaining adult height, calculated based on age corrected for gestational age at birth. Data were analyzed by linear regression adjusting for sex. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: The age at take-off, peak height velocity, and age at attaining adult height occurred earlier in VLBW subjects than in those born at term (Table).

Conclusions: Advanced timing of pubertal maturation may partly explain short final stature in people born prematurely. It may also be one of the pathways linking very low birth weight with risk factors of cardiovascular disease in adult life.

Table. Mean differences (95% confidence intervals) in ages at components of pubertal growth spurt (y) as compared with subjects born at term (n = 200) in all VLBW subjects (left column), and in those VLBW subjects who were born appropriate for gestational age (AGA) and who had no neurologic disability (right column).

	All VLBW subjects (n = 154)	p	VLBW subjects born AGA, with no neurologic impairment (n = 95)	p
Age* at take-off (y)	−0.63 (−0.91, −0.36)	0.00001	−0.69 (−1.02, −0.37)	0.00004
Age* at peak height velocity (y)	−0.38 (−0.59, −0.17)	0.0004	−0.40 (−0.65, −0.15)	0.002
Age* at attaining adult height (y)	−0.54 (−0.85, −0.23)	0.001	−0.53 (−0.89, −0.17)	0.004

*corrected for gestational age at birth.

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O-5A-37

Gene-early environment interaction and risk of Type 2 diabetes in later life

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Objective: Type 2 diabetes develops as a consequence of a genetic predisposition and unfavourable environmental circumstances. How genetic and environmental factors interact with each other remains, however, poorly undersood. A small body size at birth is one marker of a non-optimal prenatal growth. During the past years a large number of genetic loci associated with type 2 diabetes have been uncovered. The aim of the present study was to study whether there is an interaction between birth size and common variants in 9 type 2 diabetes risk genes in predicting type 2 diabetes.

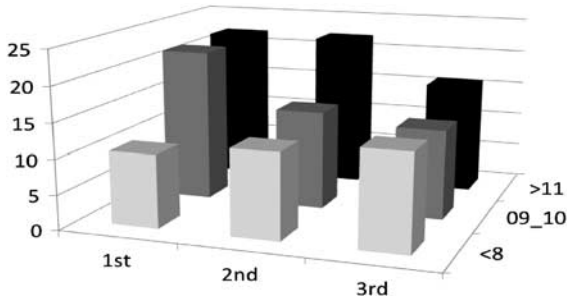
Methods: 2003 subjects participating in the Helsinki Birth Cohort Study born 1934–44 underwent a 2 hour 75 g oral glucose tolerance test (OGTT). Information on body size at birth was abstracted from hospital birth records. The participants were genotyped for common variants in the TCF7L2, HHEX, PPARG, KCNJ11, SLC30A8. IGF2BP2, CDKAL1, CDKN2A/2B and JAZF1 genes. 311 of the subjects had diabetes. Indices for insulin sensitivity and insulin secretion were calculated. Appropriate institutional

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

ethics committee clearance and participants' informed consent were obtained.

Results: Low birth was associated with type 2 diabetes ($p = 0.0008$) and impaired insulin secretion ($p = 0.04$). Pooling across all nine genes, each risk allele increased the risk for type 2 diabetes by 14%. There was a strong interaction between birth weight and number of risk alleles in predicting type 2 diabetes ($p = 0.03$). The Figure shows the prevalence of diabetes (%) according to birth weight (−3200 g/ 3200–3600 g/>3600 g) and numbers of risk alleles.

Conclusions: Conditions during fetal life, as indicated by birth weight, influence the association between risk alleles and type 2 diabetes. The association between genes and diabetes is stronger in people who were born with low birth weight.



O-5A-38

Association of maternal weight gain in pregnancy with offspring obesity, metabolic and vascular traits in childhood: the Avon Longitudinal Study of parents and children

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Objective: Greater gestational weight gain may be associated with greater offspring obesity risk and adverse vascular and metabolic outcomes, but evidence to date is scant and has relied on inadequate assessment of gestational weight gain. Our objective was to examine the association of gestational weight gain with offspring adiposity and risk of adverse vascular and metabolic traits.

Methods: Data from 5,431 (for adiposity and blood pressure outcomes) and 3,632 (for blood test results) mother-offspring pairs from the Avon Longitudinal Study of Parents and Children (ALSPAC) were used. Random effects multilevel models were used to assess incremental gestational weight gain (median and IQR number of repeat measurements per woman 12; 10–14) and in addition women were classified relative to the 2009 Institute of Medicine (IOM) recommendations (below, within or greater than recommended weight gain range). Outcomes were offspring body mass index (BMI), waist circumference (WC), DXA determined fat mass, blood pressure (BP), total cholesterol, high density lipoprotein cholesterol (HDLc), ApoA1, ApoB, leptin, adiponectin, C-reactive protein (CRP) and Interleukin-6 (IL6) assessed at mean age 9.8 years.

Results: Three distinct periods of gestational weight gain were identified: relatively low weight gain up to 14 or 15 weeks gestation (phase 1); greater weight gain from 14/15 to 35/36 weeks gestation (phase 2); levelling off of weight gain beyond 35/36 weeks (phase 3). Predicted weight at conception and gestational weight gain in each phase were positively associated with offspring BMI, WC, fat mass and SBP, with associations being robust to adjustment for confounders (offspring gender and age, maternal age, parity, smoking in pregnancy, socioeconomic position and maternal weight at conception & weight gain in the previous phase) and for potential mediation by birth weight. The magnitude of associations was strongest (when all were assessed on a standard deviation scale) for weight at conception and gestational weight gain in the second phase. Greater gestational weight gain in the second phase was associated with higher levels of offspring triglyceride, leptin, CRP and IL6 and lower levels of HDLc and ApoA1 and these associations were independent of potential confounders and mediation by birth weight. The confounder adjusted odds ratio for offspring overweight/obesity (based on age and gender specific IOTF BMI cutpoints) per 400g per week gestational weight gain in phase 2 was 1.32 (95%CI 1.13,1.53) and the equivalent result for central obesity (based on age and gender specific IDF waist circumference cutpoints) was 1.44 (95%CI: 1.26,1.65). Women who gained less than 2009 IOM recommended weight gain had offspring with reduced risk of obesity and lower average levels of BMI, waist circumference and adverse metabolic and vascular traits. Conversely, offspring of mothers who gained greater than current recommendations had an increased risk of being overweight/obese [OR 1.66 (1.41, 1.96)], centrally obese [OR 1.40 (1.21, 1.62)] and exhibited adverse metabolic and vascular traits.

Conclusion: Greater gestational weight gain, particularly in the second and early third trimester, is associated with increased risk of obesity and adverse metabolic and vascular outcomes in offspring in childhood. Appropriate institutional ethics committee clearance and participants' consent were obtained.

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

O-5A-39

Food restriction during gestation and a metabolic syndrome in later life: evidence from the Dutch Hunger Winter Families Study

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Objective: Several studies have reported inverse associations between birth weight and risk of type 2 diabetes or cardiovascular disease in later life, but few have related these observations to maternal undernutrition. We use the circumstances of the Dutch Hunger Winter of 1944-45 to assess whether maternal food availability during pregnancy was related to cardiometabolic risk in their offspring.
Methods: We studied (1) adults born in one of three hospitals in affected cities in the western Netherlands to mothers exposed to famine immediately prior to or during pregnancy (n = 359), (2) adults born in the same hospitals to mothers not exposed to famine during pregnancy (n = 299); and (3) adult non-exposed same-sex siblings of individuals in the above two groups (n = 313). We defined partially overlapping intervals of famine exposure in gestational weeks 1–10, 11–20, 21–30, or 31 through delivery based on available rations of <900 kcal/day throughout the interval. Offspring exposed in at least one such interval were considered to have "any" exposure. We variously defined metabolic syndromes as a combination of risk components (glucose intolerance, abdominal obesity, hypertension, increased triglycerides, or decreased HDL-cholesterol) using the US National Cholesterol Education Program (NCEP), the American Heart Association (AHA), and the International Diabetes Federation (IDF) criteria. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: At mean age 58 y, the overall prevalence of a metabolic syndrome was 29% (NCEP), 34% (AHA), and 44% (IDF) among the 930 study subjects with adequate diagnostic information. No gender differences in prevalence were seen for NCEP or AHA, but the IDF prevalence was higher in men (50%) than in women (38%). The age- and gender-adjusted odds ratios (ORs) for any famine exposure in relation to metabolic syndromes were NCEP: (OR 1.40; 95% CI: 1.04-1.90; p = 0.03); AHA: (OR 1.30; CI: 0.97 to 1.74; p = 0.08); and IDF: (OR 1.20; CI: 0.90–1.58; p = 0.21). OR's were consistently higher in women than in men, but p-values for all interactions by gender exceeded 0.20. For the NCEP criterion for glucose intolerance (fasting glucose >=110 mg/dl, hypoglycemic treatment or diagnosed diabetes), the OR for any famine exposure was 1.63 (CI: 1.11–2.39; p = 0.01). For all the other components of the three defined metabolic syndromes, the OR's ranged from 0.89 to 1.33, but all p-values exceeded 0.08. Associations between exposure during any specific 10-week pregnancy periods and all of the three defined metabolic syndromes yielded p-values >0.12).
Conclusions: Famine exposure in pregnancy was more strongly associated with glucose intolerance (by NCEP criterion) than other components of a metabolic syndrome.

O-5A-40

GLUT4 and *AKT1* genes are down-regulated by physical inactivity in early stages of development in rats

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Type 2 diabetes mellitus (T2DM) is a multifactorial disease and has been related to obesity as well as with levels of physical inactivity. Moreover, prior investigations have commonly associated with this condition the activity levels in adulthood. Thus, little is known about the role of physical inactivity, in early stages of development, in the predisposition for an insulin resistant phenotype. In addition, this phenotype may be related to T2DM when an adult is under the effect of a high fat diet.
Objectives: 1) To determine the effect of early physical inactivity on the dysregulation of genes involved in the glucose transport and signaling of insulin and 2) To establish the importance of the exercise lack as a cause in the increase in the susceptibility in acquiring an phenotype insulin resistant in the adulthood of subjects under the effect with a high fat diet.
Methods: Forty male Wistar rats (after birth day 23) were distributed in two groups (condition standard group (STD) and condition movement restriction (MR), in groups of 5 animals. Between days 23 and 70 after birth, MR group was kept in small cages that did not allow them to perform relevant motor activity. From day 70 to 102 after birth, 10 rats of each group were fed with hyperlipidic diet (groups STDHFD and MRHFD, 35.5% fat) and the others were fed with control diet AIN93M (STDCD and MRCD, 4% fat). During this period, all rats were kept in similar conditions. On day 103 after birth, the animals were sacrificed, and were obtained blood and soleus muscle for biochemical, physiological and molecular analysis, and furthermore were recorded the total body weight and epididymal pad fat. Plasma levels of glucose, insulin and TNFalpha were determined according to conventional techniques. Real time RT-PCR was used to evaluate the relative expression levels of GLUT4 and AKT1

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

genes. Data were quantified using value of threshold cycle, and B2M was used like housekeeping gene.

Results: Total body weight did not show significant differences between groups on day 103 after birth; nevertheless high fat diet produced a significant increase in weight of epididymal fat pad when compared to the groups fed with control diet. Differences in plasmatic levels of glucose or insulin between groups were not observed. Levels of TNFalpha were significantly higher in group MRHFD when compared to other groups ($p < 0.05$). Glucose uptake stimulated by insulin showed a significant reduction ($p < 0.05$) in MRHFD group contrasted to the other groups. Real time RT-PCR analysis showed an increase expression of GLUT4 and AKT1 gene in response to high fat diet in STD group ($p < 0.05$). Regardless of the type of diet, in the groups MR was observed a decrease in the gene expression of genes GLUT4 and AKT1 ($p < 0.05$).

Conclusions: Physical inactivity in early stages of development induces an insulin resistant phenotype when the adult subject is fed with a high fat diet. This diabetogenic response can be explained for the dysregulation of genes involved in maintaining the glucose homeostasis. Support: CONICYT-Chile.

O-5B-41

A critical look at analytical approaches to test the fetal origins hypothesis

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Objective: The hypothesis of the fetal origins of chronic diseases, also known as Barker's hypothesis or fetal programming hypothesis has gained growing acceptance, as considerable amounts of evidence has accumulated in its favor. Much of that evidence comes from observational studies in different populations. In all those studies covariant and confounding control is a difficult methodological task.[1,2]. To critically examine the methodologies to control covariation and confounding, particularly those concerning present weight or body mass index, and to prove that control for those variables can lead to biased estimates of the influence of birth weight on present blood pressure, due to suppression, a well known effect in the context of regression models.

Methods: Several simulations were carried out to reproduce different correlation patterns among birth weight, present body mass index and present systolic blood pressure[3]. Partial correlations were computed and regression models with and without covariant control were fitted to show the suppression effect. Finally, directed acyclic graph theory was used to propose a general strategy for covariant control, which can be applied in different scenarios to test the fetal programming hypothesis.

Results: Under the assumption that present systolic blood pressure is independent of birth weight, partial correlations controlling for present body mass index reverse to minus 0.04, −0.09 and −0.12, when correlations between present body mass index and present systolic blood pressure are fixed at 0.20, 0.40 y 0.50, respectively. Under the assumption that the correlation between birth weight and present systolic blood pressure is only moderately negative (−0.10) partial correlations turned out to be −0.15, −0.20 y −0.25, respectively, when correlations between body mass index and systolic blood pressure were fixed at 0.20, 0.40 y 0.50, for a fixed positive correlation between birth weight and present body mass index of 0.20.

Conclusions: (1) When studying the association between birth weight and adult blood pressure, controlling for present weight or body mass index can bias the estimation of the true association by reversing its sign from positive to negative of by making it appear more negative. (2) Covariant control in observational studies cannot be made without adequately justified theoretical hypothesis.

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O-5B-42

A new economic model of the long term costs of a poor start to life

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Objectives: This study created a new economic model to assess the potential economic effectiveness of and benefit from alternative health interventions at critical periods in the life course addressing limitations of current models. Interventions to reduce the burden of disease must be based on cost-benefit considerations[1], especially in low and middle income countries with limited health care resources. Epidemiological data show that the consequences of suboptimal intrauterine and infant conditions extend across the life course to include poor childhood health, stunting, impaired learning ability, behavioural disorders and mental illhealth, reproductive dysfunction, obesity, type 2 diabetes, heart disease, asthma and cognitive decline[2]. Current cost analyses concentrate on economic sequelae of low birth weight[3,4], without consideration of other markers of a poor start. Approaches to improving the quality of the start to life are known[5], but to date these remain largely conceptual rather than actual; maternal and infant health programmes currently focus almost entirely on immediate perinatal outcomes.

Methods: Using a human capital formation approach, we have created an analytical framework for examining the

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

impact of health on economic outcomes over the life cycle. The framework links investment in health to health state and socioeconomic factors, and economic outcomes to health states. The model has been validated for a New Zealand cohort, and scenario modelling to estimate economic costs with and without early childhood intervention has been completed.

Results: Preliminary analyses of New Zealand cohort data show the lifetime cost of a poor start in New Zealand and estimate the benefit of early childhood interventions.

Conclusions: The model provides a generic framework for estimating the lifetime health and labour costs associated with a poor start to life. It can be utilised by policy makers to make assessments of the cost effectiveness of early childhood interventions.

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O-5C-43

Placental amino acid efflux transporter mRNA levels are related to maternal diet and fetal growth

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Objective: Amino acids are vital for fetal growth, and must be supplied by the mother via the placenta. Amino acids enter the placenta across the maternal-facing microvillous membrane (MVM) of the placental syncytiotrophoblast. Previously we have shown that activity of the amino acid transporter system A in the MVM is related to maternal nutritional status. We subsequently showed that the amino acid transporters TAT1, LAT3 and LAT4 mediate efflux of specific amino acids across the basal membrane (BM) of the placental syncytiotrophoblast into the fetal circulation. This BM efflux is rate-limiting for fetal amino acid supply so may influence fetal growth. The aim of this study was therefore to investigate whether TAT1, LAT3 and LAT4 are related to fetal growth and whether they are affected by maternal nutritional status.

Methods: Tissue samples were collected from 102 human placentas from the Southampton Women's Survey. The mother's physical activity (habitual walking speed), dietary patterns and anthropometry was assessed before pregnancy, and the infant's anthropometry and body composition (dual energy X-ray absorptiometry) measured in the neonatal period. Quantitative real time reverse transcriptase PCR was used to measure the mRNA expression of TAT1, LAT3 and LAT4 normalized to the geometric mean of the housekeeping genes TOP1, UBC and YWHAZ (geNorm, PrimerDesign, UK). Pearson's correlation was used to explore the relationship between these genes and maternal and fetal factors. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: TAT1 mRNA expression was positively related to infant birth weight ($r_p = 0.22$, $P = 0.02$), head circumference ($r_p = 0.22$, $P = 0.03$) and neonatal lean mass ($r_p = 0.21$, $P = 0.03$) but not to neonatal fat mass ($r_p = 0.12$, $P = 0.25$). TAT1 mRNA expression was greatest in women from the highest social class ($r_p = 0.20$, $P = 0.04$) and with the slowest pre-pregnancy walking speed ($r_p = -0.26$, $P = 0.009$). LAT3 mRNA expression was higher in women with a lower pre-pregnancy calf circumference ($r_p = -0.24$, $P = 0.02$), and LAT3 expression predicted head circumference at birth ($r_p = 0.25$, $P = 0.01$). LAT4 mRNA expression was higher in women whose reported diets before pregnancy were less prudent ($r_p = -0.27$, $P = 0.02$). LAT4 mRNA levels were not related to birth weight or neonatal body composition.

Conclusions: TAT1 mRNA expression appears to influence fetal growth and in particular development of neonatal lean mass, but is not affected by maternal diet. High placental LAT3 and LAT4 expression in women with a lower nutritional status may reflect an adaptation in placental function to maintain the fetal amino acid supply and prevent fetal growth restriction when the maternal diet is poor. This study demonstrates how placental responses to maternal signals may influence fetal growth. This work was supported by Smith's Charity, Wessex Medical Research, the University of Southampton and by the UK Medical Research Council.

O-5C-44

Glucocorticoid receptor null mouse placentas are larger and show reduced mean harmonic thickness relative to wildype littermates

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Objectives: Glucocorticoids are essential for late fetal maturation but restrict placental and fetal growth. They act through the glucocorticoid receptor (GR), which is a ligand-dependent transcription modulator that belongs to the nuclear hormone receptor family. Homozygous deletion of the GR (GRKO) is lethal at birth due to respiratory failure[1]. Previously we demonstrated that excess glucocorticoid exposure restricts fetal growth, decreases expression of placental Vegf and reduces placental vascularisation[2]. Furthermore, reduction of endogenous glucocorticoid synthesis by metyrapone increases fetal and

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

placental weights[3], which suggests a tonic suppression of growth by physiological levels of glucocorticoids. The role of the GR in placental development, however, has not been fully defined, and so the present study investigated the effects of GR deletion on the placental phenotype.

Methods: GRKO heterozygous (+/−) mice were mated and on gestational day E18 fetuses and placentas were collected, weighed and fetuses genotyped. Unbiased stereology was used to estimate volumes of junctional (JZ) and labyrinth (LZ) zones, and LZ component volumes (trophoblast (LT), maternal blood space (MBS) and fetal capillary (FC)), surface areas of MBS and FC, and mean harmonic thickness (MHT; a measure of diffusion distance). Placental mRNA expression for peroxisome proliferator-activated receptor γ (PPARγ), 11β-HSD2, Vegfa, Igf2, Igfbp2 and the nutrient transporters SNAT-2, SNAT-4, GLUT-1 and GLUT-3, were determined by qRT-PCR.

Results: Fetal weights were comparable in the two genotypes (WT: 934 ± 70 mg, GRKO: 954 ± 62 mg) whereas placental weights were increased by 13% in GRKO placentas (WT: 73 ± 3 mg, GRKO: 84 ± 4 mg; $P = 0.004$, within-litter paired t-test). Total placental (WT: 74.7 ± 2.4, GRKO: 87.6 ± 7.1 mm^3), JZ (WT: 13.6 ± 1.9 mm^3, GRKO: 20.4 ± 3.5 mm^3) and LZ (WT: 48.6 ± 0.7 mm^3, GRKO: 53.4 ± 5.9 mm^3) volumes and LZ component volumes (except FC) were all slightly larger in GRKO placentas without reaching statistical significance. Surface areas of MBS and FC did not differ between WT and GRKO placentas, but interestingly the MHT was reduced (21%; $P = 0.02$) in GRKO placentas. Expression of 11β-HSD2 was increased (46%; $P < 0.01$) in the GRKO placentas whereas expression levels of all other genes were similar between genotypes.

Conclusions: This study shows that absence of the GR increases placental weight, consistent with the known growth inhibitory effects of glucocorticoids. Despite this, and an apparent increase in efficiency of the GRKO placenta (due to its reduced MHT), fetal weight remained unaffected.

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O-5C-45

Phenotypic outcome in offspring of mothers fed a high fat diet: a consequence of altered placental development?

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Objective: We have recently shown that moderate maternal high fat (HF) nutrition results in growth restricted offspring that develop obesity and related metabolic sequelae in adulthood independent of postnatal diet[1]. Although the underlying mechanisms are unknown, we hypothesised that maternal nutritional effects on placental function may influence fetal growth and have downstream effects on metabolic function. The present study therefore investigated the effects of a maternal HF diet during late gestation.

Methods: Wistar rats (day 100) were time mated and randomly assigned to either a control (CONT) or HF (MHF) diet *ad-libitum* throughout gestation. At e21, dams were killed; litter size as well as fetal and placental weights were recorded and maternal, fetal and amniotic samples collected. Placentas were dissected into labyrinth and junctional zones which were weighed separately.

Results: HF feeding increased maternal body weight gain during gestation (CONT 98 ± 13 g, MHF 151 ± 16 g, $p < 0.05$) and fat to lean ratio as assessed by DEXA (CONT 0.329 ± 0.05, MHF 0.739 ± 0.09, $p < 0.005$). Fetal weights were reduced in MHF male and female offspring compared to CONT ($p < 0.001$). Total placental weight was reduced in MHF pregnancies ($p < 0.05$) which was reflective of a reduction specifically in the weight of the junctional zone of the placenta ($p < 0.001$). In both nutritional groups labyrinth zone weight was reduced in females compared to males ($p < 0.05$) but was unaffected by maternal HF diet. The junctional to labyrinth weight ratio was reduced in MHF pregnancies ($p < 0.001$) and was reduced in males compared to females in both groups ($p < 0.005$). Fetal to placental weight ratio was unchanged by maternal nutrition. Maternal high fat nutrition elevated maternal and fetal plasma leptin levels compared to controls ($p < 0.005$). Maternal high fat nutrition did not alter maternal insulin levels, but fetal levels were lower in MHF female fetuses compared to controls ($p < 0.05$). Fetal insulin and leptin concentrations were positively correlated in CONT ($r^2 = 0.61$) but not MHF fetuses ($r^2 = 0.05$). Fetal glucose and amniotic fluid leptin and insulin concentrations were similar between groups.

Conclusions: These data suggest that in the rat, moderate HF nutrition results in maternal and fetal hyperleptinemia and altered placental growth and potentially function, in a sex specific manner. We speculate that these observations could in part explain previously observed sex-related susceptibility to early life nutritional programming of obesity and endocrine-related pathophysiology.

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O-5C-46

Reduced placental aquaporin water channels contribute to oligohydramnios in growth restricted fetuses

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Objective: Transplacental water flow, essential for the maintenance of fetal body water and amniotic fluid (AF), is

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

regulated by water channels (aquaporins; AQP). Maternal undernutrition (MUN) during pregnancy results in intrauterine growth restriction (IUGR) and oligohydramnios. We hypothesize that MUN impairs placental AQP expression, resulting in reduced transplacental water flow. We assessed the effect of MUN during pregnancy on maternal body weight and water intake and the resultant effects on fetal and placental weights, AF volume (AFV) and osmolality, and placental expression of AQP1 and AQP8 at select gestational ages (E13, E16, E20). We focused on two uterine positions (proximal and mid-horns) with the extremes of nutrient/oxygen supply. We also separately studied the basal placenta, site of hormone production and the labyrinth placenta, site of feto-maternal exchange.

Methods: Pregnant rats were provided either an ad libitum (AdLib) diet or were undernourished to 50% of the food of ad libitum-fed animals (MUN) beginning at E10. Maternal weights and water intake were recorded daily and at E13, E16, E20 rat dams were euthanized and gestational sacs from left and right mid- and proximal horns dissected. AFV was quantified as the difference in sac weight before and after drainage and AF osmolality determined by freezing point depression. The fetus and placenta were weighed. Six placentas from right mid- and proximal horns were separated into basal and labyrinth zones and analyzed for AQP1 and AQP8 protein expression (Western blot).

Results: Beginning at E13, MUN dams had significantly lower body weights though water intake adjusted for body weight was not significantly different from AdLib dams. At E13 and E16, MUN fetal and placental weights, and AFV and osmolality were comparable to AdLib. However at E20, MUN fetal and the placental zonal weights were significantly lower than AdLib. At E20, AFV was significantly decreased in MUN pregnancies from both mid (0.56 ± 0.02 vs. 0.70 ± 0.05 g) and proximal (0.57 ± 0.01 vs. 0.66 ± 0.04 g) horns. Conversely, AF osmolality was significantly increased in MUN mid (332 ± 5 vs. 311 ± 5 mOsm/kg) and proximal (319 ± 6 vs. 307 ± 4 mOsm/kg) horns. MUN placenta AQP1 and AQP8 protein expression were significantly lower than AdLib, as early as E13.

Conclusions: Maternal undernutrition results in reduced placental AQP1 and AQP8 water channels. We propose that oligohydramnios associated with fetal growth restriction is a result, in part, of reduced maternal to fetal water transfer.

O-6A-47

Breastfeeding duration does not affect left cardiac structures and blood pressure at the age of 2 years. The Generation R Study

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Objective: Shorter duration of breastfeeding in infancy is associated with an increased risk of development of cardiovascular disease in adulthood[1,2,3]. Early adaptations in left ventricular mass and blood pressure due to breastfeeding may explain these associations. The aim of this study was to investigate whether breastfeeding affects left cardiac structures and blood pressure development in early childhood.

Methods: This study was embedded in a prospective cohort study from fetal life onwards. Infant feeding was recorded from questionnaires at the age of 2, 6 and 12 months. Type of feeding was coded as ever breastfed or never breastfed, duration was coded as <2 months, 2-4 months, 4-6 months and >6 months. Echocardiographic measurements of left cardiac structures (left ventricular mass, aortic root diameter and left atrial diameter) and blood pressure measurements were carried out in 677 children at the age of 2 years. Weight and length were obtained at the same visit. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Never versus ever been breastfed was not associated with left cardiac structures and blood pressure at the age of 2 years. We found a tendency towards an increase of left ventricular mass and aortic root diameter in children who received prolonged breastfeeding. No associations were found between duration of breastfeeding and left atrial diameter or blood pressure. All analyses were adjusted for age, gender and current weight and length.

Conclusions: In this study, no associations of breastfeeding with left cardiac structures and blood pressure at the age of 2 years were found. Further studies are needed to investigate which mechanisms underlie the associations between breastfeeding and cardiovascular disease in adulthood.

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O-6A-48

Late gestation undernutrition programmes lipid deposition, hepatic lipid composition and fatty acid profiles in adult sheep

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Objective: To assess 1) whether late gestation undernutrition (LG-UN) have long-term implications for fat deposition, adipose tissue morphology, and hepatic lipid content and composition, and 2) whether the postnatal diet can impact on phenotypical expression of such foetal programming effects.

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

Methods: Twenty twinpregnant ewes were fed either a NORM (~ requirements for energy and protein) or LOW (50% of requirements) diet the last 6 wks of gestation (term = 147d). From 3d to 6mo post-partum (around puberty), twin lambs were assigned to each their feeding: CONV (hay) or HCHF (High-Fat-High-Carbohydrate: cream 38% fat + popped maize) supplemented with milk replacer from 3d–8wks. Male lambs were slaughtered at 6mo. Female sheep were raised on pasture from 6mo to 2 yrs (young adulthood) and then slaughtered. Hematoxylin-Eosin stainings were performed on subcutaneous fat and liver samples. Triacylglycerol (TAG), free fatty acids and phospholipids were isolated from liver lipid extract using preparative TLC. TAG content and fatty acid composition were analyzed using GLC-FID. Ceramide was isolated by solid phase extraction on NH_2-propyl cartridges and quantified as *o*-phtalaldehyde derivatives of the long-chained bases. All experimental procedures were approved by The National Committee on Animal Experimentation, Denmark.
Results: After 6 mo of differential postnatal feeding treatment, HCHF lambs became obese (BMI ~ 28–32), but LG-UN did not impact fat deposition (body weight corrected). At 2 yrs of age (after $1\frac{1}{2}$ yrs on moderate feeding), HCHF animals had lost excess body fat, but ended up having less renal and more abdominal fat compared to CONV. Adipocytes from LOW-CONV lambs (6mo) appeared immature with more extracellular matrix, and this became evident also in LOW-HCHF young adults after the loss of body fat. HCHF feeding resulted in massive accumulation of TAG ($p < 0.0001$) and ceramide ($p < 0.01$) in the liver in 6mo lambs, while the prenatal nutrition had no effect. At 2 yrs of age, TAG concentration remained higher ($p < 0.05$) in animals exposed to LG-UN, and at this age, both pre- and postnatal nutrition caused striking differences in fatty acid composition in liver lipids. LG-UN decreased C16:1 n-7 and the ratio of n-6:n-3 PUFA in phospholipids and, noteworthy, altered concentrations of fatty acids derived from rumen microbiotic activity in TAG.
Conclusions: LG-UN permanently programmed for alterations in adipocyte morphology. In lambs, it was only evident if they were not obese. Furthermore LG-UN programmed for increased baseline levels in young female adults of liver TAG and altered fatty acid composition in depot and structural lipids. Our findings interestingly suggest that gut microbiotic activity may be subjected to prenatal programming, which persists into adulthood. Changes in PUFA composition were mainly due to increased arachidonic acid and reduced docosahexaenoic acid, which could indicate long-term reduction of Δ6-desaturase activity with implications for membrane function after LG-UN in this model. Postnatal HCHF feeding caused severe liver steatosis in 6mo lambs, manifested both as increased TAG and ceramide. Long-term consequences of HCHF feeding involved repositioning of lipid deposition towards less renal and more abdominal fat in young adults. The present work was supported by the Strategic Research Council, Denmark, and The Center for Advanced Food Studies, Denmark.

O-6A-49

Early rapid weight gain and insulin resistance in young adulthood: evidence from the Cebu Longitudinal Health and Nutrition Survey

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Objective: Evidence suggests that rapid weight gain during infancy is associated with increased risk of insulin resistance and overweight in later life. Our objective was to assess the relationship between early rapid weight gain and insulin resistance in young adulthood, specifically testing whether young adult overweight mediates the relationship.
Methods: Data are from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), which follows a birth cohort to age 22 years (n = 1,532). We included non-pregnant individuals who had fasted overnight. Insulin resistance was defined as homeostatic model assessment insulin resistance (HOMA-IR) >4.65. Overweight was defined as a measured body mass index of $\geq 23\ kg/m^2$. Rapid weight gain was defined as >0.67 SD increase in weight-for-age z-score (WAZ) from birth to 4 mo. Multiple linear and logistic regression analyses were used to test the mediation hypothesis, following methods suggested by Baron and Kenny.[1] Specifically, we tested three models: 1) young adult insulin resistance was regressed on rapid weight gain, 2) young adult body mass index (BMI) was regressed on rapid weight gain and 3) young adult insulin resistance was regressed on young adult insulin resistance and young adult BMI. To establish mediation: 1) rapid weight gain must affect insulin resistance in the first model; 2) rapid weight gain must affect young adult BMI in the second model; and 3) young adult BMI must affect insulin resistance in the third model. If these conditions all hold in the predicted direction, then the effect of rapid weight gain on insulin resistance must be less in the third equation than in the first equation. In all models we included the following covariates: small for gestational age (SGA) status, urbanicity, household assets, and mother's height. In separate models we tested an interaction between rapid weight gain and SGA status. No interaction was found and this term was therefore not included in final models. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: We found no relationship between rapid weight gain during infancy and insulin resistance in young adulthood (OR = 1.33, 95% CI: 0.79, 2.20) (model 1). We found a significant relationship between rapid weight gain during infancy and young adult BMI ($\beta = 0.83$, 95% CI: 0.43, 1.23) (model 2). Finally, we found no relationship between rapid weight gain during infancy and young adult insulin

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

resistance accounting for adult BMI (OR = 1.00, 95% CI: 0.57, 1.76) (model 3), although adult BMI was significantly associated with adult insulin resistance in this model (OR = 1.38, 95% CI: 1.30, 1.46).

Conclusions: In a sample of young adult Filipinos, we found no evidence of a relationship between rapid weight gain in infancy and insulin resistance in young adulthood. It is well-known that body composition is strongly associated with concurrent insulin resistance. Effects of rapid weight gain during infancy appear to affect IR primarily through influences on young adult body composition.

1. R.M. Baron, D.A. Kenny. *J Pers Soc Psychol.,* 51:1173-82, 1986.

O-6A-50

Pre- and Postnatal Determinants of Childhood Body Size

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Objective: Growing evidence suggests obesity may have its roots in early life but it is still uncertain whether certain time periods are more critical in shaping obesity and whether critical time periods are important in predicting growth within families.

Methods: Using both a cohort approach and a sibling-based design with prospectively collected data on 20,523 participants born from 1959-1966 (10,327 boys; 10,196 girls; 571 male sibling sets; 651 female sibling sets) of the National Collaborative Perinatal Project (NCPP), we investigated the associations between pre- and postnatal factors and childhood body size at 7 years. We compared quantile regression to linear and logistic regression models. We further examined associations with prenatal factors using time-varying quantile regression approaches.

Results: Maternal body mass index (BMI), maternal pregnancy weight gain, birth weight and postnatal weight change for three time periods (birth-4 months; 4–12 months; 1–4 years) were all positively and independently associated with BMI at 7. The impact of postnatal weight change on BMI at 7 was similar for each time period. For example, a 10 percentile increase in weight increased the probability of being overweight at 7 years by approximately two-fold regardless of time period (OR = 1.8–2.1 for boys and girls); associations were strongest for the upper quantiles of childhood BMI. Analyses of differences between same-sex siblings also revealed important and independent associations between maternal BMI, pregnancy weight gain, and infant and early childhood weight gain on childhood BMI at 7 years. In particular, we observed, statistically significant associations between maternal pregnancy weight gain and maternal BMI among the upper quantiles of BMI differences for the within-sibling analyses. Finally, in analyses applying time-varying quantile regression approaches, we observed strong associations between maternal pregnancy weight gain and maternal BMI among the upper quantiles of BMI at 7 years.

Conclusion: These consistent findings both from the overall cohort and the sibling analyses suggest that there are multiple, rather than specific critical periods of influence shaping childhood body size and that prenatal and early infant factors are important predictors of body size even within same-sex siblings.

O-6B-51

Low protein diet *in utero* results in reduced ureteric branching in cultured metanephroi, but the renal renin-angiotensin system is not involved

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A maternal protein restricted diet is frequently associated with increased blood pressure, an altered renin-angiotensin system (RAS) and reduced nephron number in adult offspring. Despite the ubiquity of this model there is uncertainty as to when in development these effects manifest. In rats, development of the permanent kidney (metanephros) begins around embryonic day 13 (E13) when the ureteric bud invades the metanephric mesenchyme. Through a process of reciprocal induction, the ureteric bud branches forming the ureteric tree, while the mesenchyme condenses forming nephrons. Nephron endowment is dependent on both processes. The RAS is involved in this process and also regulates adult cardiovascular and renal function. We hypothesise that altered levels of expression of RAS elements during nephrogenesis contribute to the phenotype observed in offspring of protein-restricted rats.

Objective: To investigate the effects of maternal low protein diet exposure *in utero* on ureteric branching morphogenesis and RAS expression during gestation.

Methods: Virgin female Sprague- Dawley rats were fed for 2 weeks prior to mating and during pregnancy a normal-protein diet (control – 20% casein) or protein-restricted diet (LP 8% casein). Metanephroi (n = 6) were obtained from male offspring of time-mated rats at embryonic day 14.5 (E14.5) and cultured in defined media at the air-media interface (37°C, room air with 5% CO_2). After 48 hours, metanephroi were fixed, immunostained (monoclonal-anti-pan-cytokeratin 1°antibody, Sigma-Aldrich) for visualization of the ureteric tree and quantification of ureteric branch points and ureteric tips. mRNA expression of renin, angiotensinogen and angiotensin II receptors (AT1Ra,

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

AT1Rb and AT2R) was measured (n = 8) by real time-PCR at E15.5, E17.5 and E20.5 and 24 h hours after birth (PN1) using ribosomal 18S as the endogenous control. Data are presented as mean ± SEM and n refers to animals sourced from separate litters.

Results: Metanephroi cultured from offspring of LP rats had significantly fewer ureteric branching points (28.93 ± 2.68 vs control 39.78 ± 0.578, p = 0.02) and terminal tips (31.35 ± 2.63 vs. control 41.13 ± 1.39, p = 0.03) than controls. Expression of renin, angiotensinogen, and AT1Ra mRNA was similar in control and LP offspring at E 15.5 and E17.5. At E20.5 renin expression was augmented in LP offspring (2.38 ± 0.42 vs control 1.06 ± 0.16 p < 0.01). Angiotensinogen expression was augmented in LP offspring (3.43 ± 0.64 vs control 1.20 ± 0.54, p < 0.05) at PN1 as was AT1a (3.06 ± 0.79 vs control 1.04 ± 0.14, p < 0.05). In LP rats AT1Rb mRNA was down-regulated at E17.5 (0.63 ± 0.1 vs control 1.08 ± 0.15, p < 0.05) but augmented after birth. ATR2 expression was similar between groups.

Conclusion: This study is the first to identify that exposure to a LP diet disrupts normal kidney development from early in kidney development. This 25% reduction in ureteric tree branching is consistent with the 20–30% reduction in nephron endowment often observed in adult offspring. Changes in expression levels of RAS elements are consistent with adult cardiovascular dysfunction but appear to occur later in development than the initial change in branching morphogenesis. Grants from CNPq, CAPES and FAPESP supported this work.

O-6B-52

Cardiovascular responses to dietary salt-loading in adult male and female offspring of fat-fed rats

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Objective: Increased maternal dietary fat intake in rodents can adversely affect cardiovascular health of the progeny in the absence of maternal obesity. Evidence suggests that altered sympathetic control of cardiovascular function may arise in offspring subjected to nutritional imbalance *in utero*. In this study we hypothesized that a maternal diet rich in saturated fat, may influence the autonomic response to a high-salt diet in adult progeny which may contribute to cardiovascular dysfunction.

Methods: SD dams were fed either control diet (5% fat) or diet enriched with 25% lard throughout pregnancy and lactation. Litters were standardised and weaned onto a normal diet. Telemetred 9-month old offspring were given the standard control diet enriched with 8%-NaCl *ad libitum* for 1 week and then replaced with the standard chow. Cardiovascular parameters were recorded for one week at baseline; one week of salt-loading and one week of washout to compare responses to salt-loading between offspring from control and fat-fed dams. Heart rate variability was analysed using the HRV module of Chart 5.0 software (ADInstruments Pty Ltd, Australia). Kidney and plasma noradrenaline were determined by ELISA, using an EIA kit (ALPCO Diagnostics, USA).

Results: There were no differences in body weight gain or food intake either in dams fed a standard chow or a high-fat diet or in their progeny. Baseline systolic (SBP) and diastolic blood pressure were similar in male offspring of control (OffCon) and fat-fed dams (OffLARD). During salt-loading male OffLARD exhibited significantly elevated night-time SBP Versus OffCon (F = 6.5; P = 0.038, RM ANOVA). These effects were reversed during the washout period. Females did not demonstrate any differences in SBP. Heart rate was unaffected by salt-loading and was similar in OffLARD and OffCon. HRV analysis performed on a last day of salt-loading showed that ratio of low frequency to high frequency (LF/HF ratio) of power spectrum was increased in male OffLARD (male OffLARD, 0.65 ± 0.11 *Versus* male OffCon, 0.37 ± 0.067 P = 0.049) although LF and HF spectral bands were comparable between the two groups. There were no differences in LF/HF between female OffLARD and OffCon following salt-loading although LF was significantly increased in female OffLARD (6.9 ± 1.04 ms^2 vs 3.28 ± 0.06 ms^2 OffCon, P = 0.02) and HF remained similar between two groups. Basal renal noradrenaline content was elevated in male OffLARD relative to controls (male OffLARD 5.35 ± 0.36 *vs* male OffCon 4.34 ± 0.24 $pg\,ml^{-1}\,mg\,tissue^{-1}$ $P = 0.04$).

Conclusions: Exposure to a high-fat diet *in utero* results in hyper-responsiveness of SBP to a dietary salt load in adult male offspring but not in females. The salt-induced elevation of renal noradrenaline and increased LF/HF ratio suggests an increased renal sympathetic outflow in response to salt-loading. A maternal diet rich in animal fats therefore appears to affect autonomic pathways regulating the blood pressure response to a salt load in male rats. This may contribute to a gender-specific increased susceptibility to cardiovascular disease in adult life. Support: The British Heart Foundation Grant No PG/06/081/21195.

O-6B-53

Maternal folic acid deficiency compounds the renal developmental anomalies induced by exposure to a low protein diet

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https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

Maternal folate intake promotes beneficial birth outcomes. Although the mechanism is unclear, antioxidant properties, a role in cell cycle regulation and the ability to modulate gene methylation appear important. *In utero* growth restriction in humans and maternal protein restriction in experimental models result in lowered kidney nephron number which may affect adult cardiovascular health. It is not known whether folate supplementation can alter kidney development. Using an organ explant culture system, we have observed reduced ureteric branching morphogenesis in offspring of protein-deprived rats from embryonic day 14.25 (E14.25) (Mesquita *et al* unpublished). We hypothesise that folate supplementation, to the maternal diet or to an explant culture system may counteract the effects of a maternal low protein diet.
Objective: To determine if dietary embryonic kidney development is affected by maternal dietary folic acid supplementation or deficiency and whether application of folic acid in an *ex vivo* culture system can normalise reduced kidney growth initiated by a low protein diet.
Methods: Female Sprague-Dawley rats (n = 4–6 per group) were fed one of 3 maternal diets for 3 weeks and then time mated (3 h). All diets (Glen Forrest Stockfeeders, Western Australia) were low in protein (9% casein) to induce a nephron deficit. Various concentrations of folic acid, 2 mg/kg (control LP), 200 mg/kg (LP+) or <0.05 mg/kg (LP−), were used to investigate the rescue potential of folate. Rats remained on their respective diets until E14.25 when embryonic kidneys were dissected and cultured for 48 hours in a serum-free defined media (DMEM:HamsF12) containing 0, 0.6 or 2mM folic acid (Sigma Aldrich). Cultured kidneys were immunostained for cytokeratin to visualise the ureteric epithelium. Branching morphogenesis (branch points and terminal tips) was quantified. Data were compared by 2-way ANOVA with maternal folate status and culture folate supplementation as independent variables. Data are presented as mean ± SEM.
Results: Overall, maternal folic acid status ($P < 0.002$) but not culture folic acid status ($P = 0.17$) affected branching morphogenesis in the kidney. There was no interaction between maternal folate intake and culture folate supplementation ($P = 0.64$). In media without added folate, maternal protein and folate deficiency resulted in fewer branch points than low protein alone (LP−26.6 ± 6.0 n = 5 vs. LP 46.3 ± 3.3 n = 6, $P < 0.01$) and tips (LP−27.6 ± 6.0 n = 5 vs. 47.5 ± 3.2 n = 6, $P < 0.01$). Addition of folate (LP+) to the maternal diet did not result in an increase in ureteric branches (41.0 ± 6.0 n = 4, P = 0.47) or tips (42 ± 6.2 n = 4, P = 0.46) when compared with LP alone.
Conclusions: Maternal folate restriction superimposed on maternal protein restriction exacerbates the reduction in kidney branching morphogenesis. Maternal folic acid supplementation does not rescue the phenotype induced by exposure to a low protein diet. Addition of folate to culture had no effect on branching morphogenesis, suggesting that the programme of branching morphogenesis may be determined prior to kidney culture at E14.25 or that additional co-factors are required in a serum-free culture system. Support: National Heart Foundation of Australia Fellowship (PF 06M-2766) and Monash Fellowship to JAA.

O-6B-54

Preterm birth is associated with accelerated renal maturation and abnormalities in glomerular morphology

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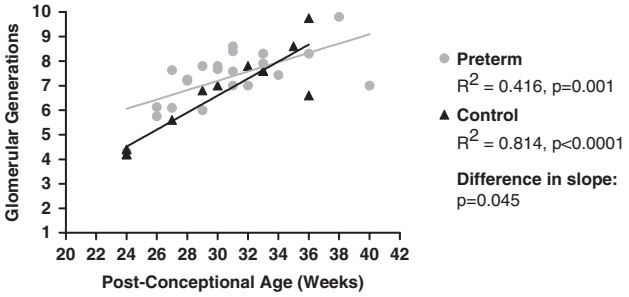
Objective: Nephrogenesis, the formation of nephrons in the kidney, is complete by 36 weeks gestation with 60% of nephrons formed during the last trimester. Following preterm birth, nephrogenesis continues postnatally, however, it is unknown whether nephrogenesis follows the normal developmental trajectory as that *in utero*. The aim of this study, therefore, was to determine the effects of preterm birth on nephrogenesis.
Methods: Archived renal autopsy tissue from preterm neonates (born at 24–34 weeks gestation; 2–36 days postnatal survival) with no evidence of growth restriction (n = 25) was compared to renal autopsy tissue from stillborn neonates (24–33 weeks gestation) that died acutely *in utero* (n = 12). Stereological analysis was undertaken to determine glomerular generation number, glomerular maturity, and the percentage of abnormal glomeruli within each kidney. Image analysis software was utilised to calculate average renal corpuscle size and nephrogenic zone thickness. Appropriate institutional ethics committee clearance and informed parental consent were obtained.
Results: The number of glomerular generations formed per week of post-conceptional age was significantly reduced in the preterm group compared to the controls (Control: 0.35 ± 0.05 generations/week; Preterm: 0.19 ± 0.05 generations/week; p = 0.045), as shown in the figure below.



Renal corpuscle size was significantly increased in the preterm group compared to the controls (Control: 4,474 ± 236 μm^2;

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

Preterm: 5,475 ± 174 μm^2; $p = 0.004$). The percentage of abnormal glomeruli (with an enlarged Bowman's space and underdeveloped tuft) present in the outer renal cortex of the preterm kidneys ranged from 0% to 8.6%, and was significantly greater than the percentage of abnormal glomeruli present within the control kidneys ($p = 0.01$). No significant difference in nephrogenic zone thickness was found between the two groups. Importantly, however, there was a significant decrease in the percentage of glomeruli at the most immature (vesicle) stage of development within the preterm kidneys compared to the controls ($p = 0.02$).

Conclusions: The findings of this ongoing study suggest that preterm birth leads to accelerated renal maturation. Furthermore, both the reduced number of glomerular generations formed *ex utero* and the abnormalities in glomerular morphology indicate that preterm birth may lead to impaired renal function, both in the neonatal period and later in life. Support: National Health and Medical Research Council of Australia (NHMRC).

O-6C-55

The influence of folate and vitamin B_{12} status and folate-related genotype on the transmission of DNA methylation patterns from mothers to offspring

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Objectives: The transmission of DNA methylation patterns from parents to offspring has important implications for the heritability of both innate and environmentally acquired epigenetic marks. It is well-established that imprinted regions of the genome are protected from comprehensive demethylation post fertilisation, but little is known about the mechanisms that 'protect' non-imprinted loci against demethylation or that make loci particularly susceptible to remethylation.

Methods: Infants and pregnant women were drawn from the North Cumbria Community Genetics Project; a biological sample bank composed of an unselected population based cohort of 7,000 pregnancies. Red blood cell folate (RCF) and serum vitamin B_{12} levels were measured in antenatal blood samples and data collected on smoking and other exposures during pregnancy by questionnaire. DNA samples from 412 infants (cord blood) and 326 pregnant women (peripheral blood) were analysed for folate metabolism polymorphisms. DNA methylation was quantified at CpG sites in an imprinted gene (*IGF2;* 3 CpGs) and two non-imprinted genes (*IGFBP3;* 5 CpGs, *ZNT5*; 5 CpGs) in the same subjects using Pyrosequencing. Methylation data were skewed and were analysed using non-parametric statistical tests. Mean methylation levels at each locus were analysed in relation to exposure (maternal and infant red cell folate and serum B_{12}, maternal age, paternal age, smoking, genotype) and outcome variables (birth weight, gestational age). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Methylation levels across multiple sites within *IGF2* and *IGFBP3* were highly correlated (coeff; 0.86 to 0.99, $p < 0.0001$). In contrast, methylation levels for the 5 CpGs in *ZNT5* were less well correlated. Gene-specific methylation showed poor correlation with genome-wide methylation at all CpGs studied. In general, methylation levels between mothers and their infants were poorly correlated. RCF status was correlated positively with methylation of *IGFBP3* in mothers (coeff; 0.148, $p = 0.022$) but this correlation was not observed at any other loci or at a genome-wide level in mothers or infants. Maternal vitamin B_{12} levels were positively correlated with genome-wide methylation in infants (coeff; 0.175, $p = 0.028$). Maternal smoking during pregnancy was associated with higher genome-wide methylation in infants (t-test; $p = 0.043$). Maternal age, paternal age, birth weight and gestational age had no detectable effects on infant methylation status. Maternal genotype suggested that several folate-related SNPs exert modest effects on gene specific methylation in mothers. In all instances the presence of the variant was associated with lower gene-specific methylation. Child genotype for folate-related genes had different effects on child methylation status for each gene. The *MTRR* 66G>A variant was also associated with elevated genome-wide methylation. Maternal genotype was associated with infant methylation status with the *MTRR 66G>A* variant showing the most pronounced effect.

Conclusions: Collectively these data provide some evidence that variation in folate and vitamin B_{12} availability and metabolism can influence not only DNA methylation levels in the individual but also the transmission of methylation patterns from mother to offspring. The data point most consistently to a role for vitamin B_{12} in this process. Support: NuGO (EU FP6 Network of Excellence) for funding the Nutritional Epigenomics Focus Team.

O-6C-56

Gene-environment interactions between the fat mass and obesity-associated (fto) gene and nutrition modify the association between Fto and obesity in childhood and adolescence

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https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

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Objective: We have recently shown that the AA genotype in the SNP rs993960 in FTO is associated with symmetric intra-uterine growth restriction (male and female) followed by obesity during adolescence in males. The aim of this study was to investigate gene-environment interactions between the FTO gene and nutrition and exercise in the Western Australian Pregnancy (Raine) cohort to identify potential interventions to minimise the impact of the FTO gene on obesity.

Methods: Pregnant women were recruited at 16-weeks gestation (n = 2900) and carefully phenotyped during pregnancy and childhood including assessment of anthropometry, nutrition, activity/fitness and socio-economic status (SES). The amount and frequency of consumption of 212 foods was collected at 14-years and data were collapsed into 38 food groups; a maximum likelihood factor analysis was conducted. Two independent patterns ('Prudent/Healthy' diet and 'Western' diet) were identified. The rs9939609 SNP was genotyped in 1079 children with Caucasian parents. Associations between rs9939609 and BMI at 8-, 14- and 17- years were investigated using multivariate linear regression. Gene-environment interactions involving sex, post-natal nutrition and activity were explored. Institutional ethics committee clearance and participants' informed consent were obtained.

Results: BMI at 8-, 14- and 17-years was associated with birth-weight, maternal smoking during pregnancy, maternal education/employment, and family income (all $p < 0.05$). The rs993960 AA genotype was independently associated with increased BMI in males (but not females) at 8- ($p < 1 \times 10^{-5}$), 14- ($p < 1 \times 10^{-5}$) and 17-years ($p = 0.001$) in multivariate analyses adjusted for birth-weight, smoking, SES, nutrition and activity. Breast feeding was associated with reduced BMI in childhood/adolescence, in males but not females, independent of FTO genotype ($p \leqslant 0.001$). In both sexes increased weight gain over the first year was associated with higher BMI in adolescence (all $p < 1 \times 10^{-5}$). The AA genotype was associated with greater weight gain in the first year compared to TT. Further, AA males at 14-years with normal BMI had less weight gain in the first year than AA males who were overweight/obese at 14. A 'Western' dietary pattern was not associated with increased BMI in females until 17-years ($p = 3 \times 10^{-4}$). Increased duration of physical activity was associated with a decrease in BMI at 13- ($P = 0.01$) and 17-year ($P = 0.02$), independent of FTO genotype. Gene-environment interactions were identified between FTO and measures of nutrition. The impact of weight gain in the first year of life on BMI at 8-, 14- and 17-years was greater in AA than TT genotypes ($p = 0.03$). Further, the reduction in BMI at 8 and 14-years in males associated with breast feeding was greater in AA than TT genotypes. There was a significant interaction between FTO genotype and diet at 17-years in females, with an even greater reduction in BMI seen in AA than TT genotypes ($p = 6 \times 10^{-4}$) in those with a prudent/healthy diet.

Conclusion: We have found evidence of gene-environment interactions between childhood nutrition and the FTO gene acting of measures of childhood obesity. These findings highlight the complex pathways underlying the development of childhood obesity and raise the possibility of reducing the prevalence of this condition by targeted interventions in those at greatest genetic risk.

O-6C-57

The fat mass and obesity-associated gene, a genetic marker of fetal programming?

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Objectives: Numerous epidemiological data using genome wide analysis have found genetic variants of the fat mass and obesity-associated (FTO) gene to be conclusively related to obesity in various human populations[1,2]. This gene is widely expressed within tissues and FTO has been proposed by Gerken and colleagues[3] to participate in the control of appetite via unknown mechanisms. Our objective was to investigate whether the FTO gene, as a gene candidate for obesity, could be modified by maternal nutrition and, therefore, represent a new target for the development of obesity.

Methods: Pregnant twin-bearing sheep were either fed a control (C; n = 8) or a nutrient restricted diet (NR; n = 8) from early to mid-gestation (30 to 80 dGA) and a control diet thereafter. Mothers gave birth naturally at term to twins. One of the twin offspring was humanely euthanased at 7 days of age and its sibling kept with its mother up until weaning. Thereafter, animals were raised in a control indoor environment up to 12 months of age. At both ages, entire hypothalamus, heart, kidneys, liver, adipose tissue depots and skeletal muscles were collected, snap frozen and kept at −80°C for further analysis of FTO gene expression. At 12 months of age, average food intake was determined by collecting food intake measurements over a two week period and triglyceride content in cardiac muscles was determined by the Folch method. Appropriate institutional animal ethics committee approval was obtained.

Results: All offspring had similar body weights at birth and exhibited similar growth rates throughout the first year of life. In the newborn, no differences in FTO gene expression were found in any of the tissues analysed. However, at 1 year of age, offspring born to NR mothers exhibited overexpression of hypothalamic FTO as compared to controls ($p = 0.02$), a relationship linked with a substantial reduction in energy

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

intake ($p < 0.05$). In addition, these offspring exposed to in utero NR exhibited a down regulation of FTO in cardiac muscle ($p < 0.01$), a modification correlated to an increase in cardiac triglyceride content ($p < 0.05$).

Conclusions: When alterations of the fetal energetic environment are targeted during the period of early hypothalamic and cardiac organogenesis, they both exhibit substantial changes in FTO gene expression. Interestingly, we observed these adaptations changes to be related to physiological alterations (i.e. food intake and triglyceride uptake). These findings therefore support a role for FTO in the control of energy metabolism, suggesting FTO as a gene target for fetal programming. Unfortunately, very little is currently known about FTO functions *in vivo* and we are investigating further its biological roles.

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O-6C-58

The importance of being accurate: gestational age and reported associations between gene:environment interactions and birth weight

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Objective: Genome-wide association studies (GWAS) are revolutionising the search for the genes underlying gene:environment (G:E) interactions in early life. Recent data have demonstrated G:E interactions between smoking, polymorphisms in CYP1A1, GSTT1, GSTM1 and FTO and intrauterine growth restriction. Interaction analyses between common environmental exposures and common genetic variants thus have a growing role in early life investigations of chronic disease etiology. It is critical to account for gestational age (GA) in analyses of birth weight (BW). This research assessed the impact of the accuracy of GA estimation on the ability to detect associations of complex gene:environment interactions with BW using an exceptional birth cohort resource with extensive antenatal data.

Methods: The Western Australian Pregnancy (Raine) Study recruited unselected women from the general population at 16-18 weeks gestation. Appropriate institutional ethics committee clearance and participants' informed consent were obtained. Analyses focused on 2,065 full-term, singleton-births. GA at birth was recorded (1) based on the date of the last menstrual period (LMP) alone and (2) based on extensive ultrasound biometry throughout pregnancy. Multivariate linear regression models of BW were constructed, including parity, sex, maternal smoking and GA as covariates. G:E power calculations were based on 80% power, a MAF $\geq$ 5%, a continuous environmental exposure, a dominant genetic model, and an α of 10-4.

Results: GA based on the date of LMP alone and based on ultrasound biometry accounted for 7% and 16% of the total phenotypic variance in BW, respectively. The correlation between the two methods of GA measurement was 0.69. Using LMP-estimated GA alone, studies $\sim$10% larger would be required to detect a true association between BW and a gene:environment interaction compared to ultrasound-estimated GA. In terms of precision of a continuous outcome measure, decreases in BW or GA precision across larger studies would have an even greater impact on power.

Conclusion: Our results suggest that smaller birth cohort studies with more precise measures of gestational age will be as powerful as larger studies in detecting gene:environment interactions for continuous traits. These findings should be helpful when designing or interpreting analyses of pregnancy cohorts which have gestational age based only upon date of last menstrual period.

O-7A-59

In the associations between body mass index, plasma glucose and birthweight: Is there a mediating role of interleukins IL-6 and IL-1Ra?

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Objective: The biological mediator mechanisms behind the well known association between maternal body mass index (BMI) and birthweight are not well understood. Maternal body composition is associated with plasma glucose level and nutrient transport across the placenta, which are central regulators of fetal growth. Interleukin 6 (IL-6) and Interleukin-1 Receptor antagonist (IL-1Ra) can be produced by adipose tissue and there is evidence of a role of these markers in carbohydrate metabolism. In addition, preliminary data suggest that placental transport capacity may be affected by interleukins[1]. We hypothesized the following main pathways through which adipose tissue can affect birthweight: The direct or unexplained pathway, and the indirect paths involving the production of interleukins and the level of fasting glucose (nutrient availability). The mediating role of interleukins was divided into direct effects on birthweight and indirect paths through fasting glucose (see

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

figure). We wanted to quantify the contributions made by these interleukins and glucose on birthweight.

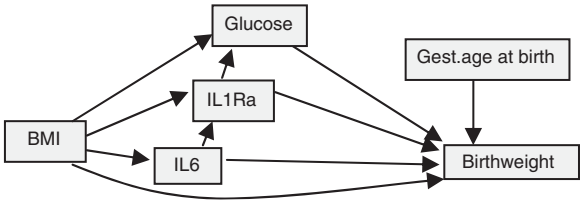

Figure: Path Model (DAG).

Methods: Fasting glucose, IL1-Ra and IL-6 were obtained from n = 240 normal pregnant women at gestational week 30–32. BMI was recorded in gestational week 14–16. A Directed Acyclic Graph (DAG) was specified to incorporate known and hypothesized associations between the variables. Direct effects and total effects of BMI, IL-1Ra and IL-6 on birthweight were estimated by standard multiple linear regression and Bayesian path analysis (WinBUGS). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Mean BMI for the cohort was 25.0 (SD 4.2), 48% were primipara and mean birthweight 3744 (SD 448). There was a significant association between early pregnancy BMI, and IL-6, IL-1Ra and fasting glucose in third trimester. The adjusted effect estimates obtained from regression analysis showed significant effects of BMI and glucose on birthweight, whereas the effects of IL-1Ra and IL-6 on birthweight were statistically non-significant. However, the model showed a 41% increase in the effect of BMI when considering indirect pathways through glucose, IL-1Ra and IL-6, as compared to the adjusted direct effect obtained from standard multiple regression analysis. The corresponding standardized regression coefficients for the direct effect was 0.17, CrI [0.01–0.32], and for the total effect 0.24, CrI [0.12–0.37]; all effects corrected for gestational age at delivery.

Conclusions: Given the DAG specified in the present work, there is evidence for a mediating effect of inflammation on birthweight. In the present model, almost 70% of the total effect of BMI on birthweight went through unexplained pathways, approximately 15% via glucose only and 14% via paths involving IL-1Ra.

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O-7A-60

Pre- and postnatal nutrition of infants with obese mothers: review of metabolic imprinting and later health outcomes*

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Objective: The prevalence of obesity in pregnancy is growing with the overall obesity epidemic. Although there is abundant evidence about the deleterious effects of excessive body weight and fat mass on metabolic and physiological processes within the body in the non-pregnant state, there is little known about these influences during pregnancy and lactation on the health of both the infant and the mother. Maternal overweight and obesity is associated with dysregulation of metabolic, vascular and inflammatory pathways and they have been identified as significant risk factors and with childhood overweight. The objective is to map out the research landscape, compile a cohesive review of available evidence and develop science-based guidelines on pre- and postnatal nutrition of infants born from overweight and obese mothers in relation to later health.

Methods: The compilation, review and analysis of evidence is based on the identification of functional mechanisms such as nutrient supply, the applicability of translational research and methodology and the identification of short- and long-term health consequences and knowledge gaps in this area. The outcomes were evaluated and discussed by more than 20 scientific experts during a recently organised workshop in Brussels, and intended to be published in a peer-reviewed scientific journal.

Results: The following themes were evaluated and documented: strength of evidence from both human epidemiological and animal studies, role of maternal nutritional status, tools for accurate assessment of maternal nutritional status in overweight and obese pregnancies, role of maternal insulin resistance and gestational diabetes, infant growth and body composition *in utero* and postnatally, role of nutritional status during early life, mechanisms including maternal and neonatal dietary factors and placental function, interventions in obese pregnancies, rationale of current strategies including weight gain restriction and dietary and physical/behavioural changes, identification of sensitive and dose-responsive biomarkers with long-term efficacy, and evidence for epigenetic and transgenerational effects.

Conclusions: This review provides a scientific framework for optimising nutrition in which guidelines in particular for pregnant and lactating overweight and obese women are developed, with potential significance to all overweight and obese women of child-bearing age. The review provides also a foundation to guide future research in this area.

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

O-7A-61

Obesity related hypertension, increased sympathetic drive and selective leptin resistance are programmed by exposure to a fat-rich diet during development

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Introduction: Maternal obesity or consumption of diets rich in saturated fatty acids during pregnancy can programme obesity related hypertension in the offspring but the aetiology is unclear. Leptin, secreted from white adipose tissue in proportion to its mass, acts at nuclei in the hypothalamus to decrease food intake and increase renal sympathetic nerve activity (RSNA), resulting in increased blood pressure. Selective leptin resistance; resistance to the anorectic but not pressor effects of leptin is thought to occur in adult obesity. It is not known, however, if selective leptin resistance or altered sympathetic drive can be programmed by maternal dietary challenge.
Objective: To determine if offspring from mothers fed fat rich diets in pregnancy and suckling become obese and hypertensive and to determine if these offspring demonstrate evidence of selective leptin resistance and increased RSNA.
Methods: Female New-Zealand white rabbits were fed either a control (3.5% fat from soy oil) or high fat diet (HFD, 13.5% fat from lard and soy oil) 3 weeks prior to mating, throughout gestation and lactation. Offspring were meal fed a control diet after weaning (6 weeks), effectively normalizing the post weaning caloric intake in all animals. At 4 months of age all rabbits were instrumented with intracerebroventricular (*icv*) guide tubes to the lateral ventricle and electrodes on the renal nerve. Blood pressure was measured by direct cannulation of the central ear artery. Basal haemodynamics and RSNA were measured then the responses to *icv* injections of leptin (5, 10, 50 and 100 μg) assessed. The anorectic response to leptin was measured by comparing 24 hour food intake after an injection of 100 μg leptin or vehicle. These studies were approved by the local animal ethics committee.
Results: Body weight was similar between groups, however HFD offspring (n = 9) had ~40% heavier visceral white adipose deposition compared with controls (n = 8, $P < 0.05$) despite having a similar caloric intake post-weaning. HFD offspring demonstrated elevated basal blood pressure and RSNA compared with controls ($P < 0.05$). HFD offspring showed augmented pressor responses (ΔBP 4.8 ± 1.0 mmHg vs 2.6 ± 1.0 mmHg, $P < 0.05$) and renal SNA responses ($P < 0.05$) to *icv* leptin compared with controls. HFD offspring showed blunted anorectic effects to leptin compared with controls (−8.2 ± 4.8 g vs −21.3 ± 4.6 g, $P < 0.05$).
Conclusions: Exposure to a fat rich diet in development programmes increased adiposity even with a calorie controlled diet, indicating programmed changes to metabolism. These are the first data to show that offspring from mothers consuming a HFD show selective leptin resistance. This selective leptin resistance appears to result from alterations in hypothalamic integration of appetite and cardiovascular control and may contribute to the development of increased adiposity and elevated blood pressure in these offspring. Further investigation as to the location and neurochemical signature of affected neurons is now warranted. *Support:* This work was funded by a National Heart Foundation of Australia Fellowship (PF 06M- 2766), Monash Fellowship and Baker grants to JAA.

O-7A-62

Evidence of fetal insulin sensitivity programming by maternal glucose tolerance in humans

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Objective: Infants born to mothers with impaired glucose tolerance are more likely to develop insulin resistance-related disorders in later life. Plausibly, such "programming" effects may be mediated through altered fetal insulin sensitivity. However, there remains an absence of biochemical proof of such intrauterine programming in humans. We assessed the association between maternal glucose tolerance during pregnancy and fetal insulin sensitivity.
Methods: We conducted a hospital-based prospective pregnancy cohort study (n = 152). Pregnant women bearing a singleton fetus without pre-existing diabetes were recruited at middle (24–27 w) gestation, and followed up at 32–35 w of gestation and delivery. Maternal blood samples were collected at 24–27 w at the time of the 1-h 50 g oral glucose challenge test. Venous cord blood samples were obtained immediately after birth. Glucose (mg/dl)/insulin (μU/mL) ratio was used as an indicator of insulin sensitivity. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: The median (inter-quartile range) of glucose (mg/dl)/insulin (μU/mL) ratios were 2.3 (1.5, 3.3) in maternal blood samples at 24–27 w of gestation during the 1-h glucose challenge test, and 21.6 (12.6, 31.6) in neonatal cord blood samples. Higher maternal blood glucose levels in the 50 g glucose challenge test were associated with significantly lower fetal insulin sensitivity (glucose/insulin ratios) ($r = -0.32$, $P < 0.0001$). In patients with impaired glucose tolerance (glucose > 7.8 mmol/L or 140 mg/dl in the 1-h glucose challenge test result), fetal insulin sensitivity was significantly impaired (n = 29, median glucose/insulin ratio = 16.2) as compared to patients with normal glucose tolerance (n = 123, median glucose/insulin ratio = 24.5) (Kruskal-Wallis test for differences: P = 0.02).

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

Conclusions: These data indicate that maternal glucose tolerance in pregnancy may program fetal insulin sensitivity, and consequently may "program" the long-term susceptibility to the metabolic syndrome and related disorders in later life. Acknowledgements: Supported by two research grants from the Canadian Institutes of Health Research (CIHR), Institute of Nutrition, Metabolism and Diabetes (INMD) (Dr Luo), and Institute of Human Development, Child and Youth Health (IHDCYH) (Dr Fraser).We were indebted to all dedicated research nurses, assistants and technicians for their excellent work in the project, and to all study patients for their invaluable contributions.

O-7B-63

A theory of global health

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A life expectancy disparity of over forty years separates the healthiest and least healthy nations in the world today, an all time high. The least healthy countrys are no better off than societies in the neolithic despite millennia of progress. Why do countries end up with the health they? Operative factors include how nations satisfy essential needs of food, clean water and shelter. After that the structure of early life is important as is the nature of caring and sharing there. Intergenerational transmission of health requires a historical perspective that will be presented. Colonialist history had three major types, leading to three clusters of nations today with health outcomes among the best to those among the worst. These included parts of the world where colonists came, enabled the local elites to plunder their people and the colonists did settle in substantial numbers because conditions were harsh. After independence, the local elites continued their extractive processes and poor health outcomes continued. An intermediate group was where colonists came and settled in the minority and ruled after independence. The third group of countries, all English speaking, were those where the colonists came, decimated the local population and ruled as the majority. Their health outcomes were on a par of the nations from whence they came. Critically important is when health started improving in the last few centuries, and cultural factors operate as well. Health care is not found to be an important factor. The theory suggests that for today's record disparities to come together will require far sighted plans that try to redress the exploitation of colonialism and to not continue modern day policies of resource extraction. Long term provision of essential needs and primary health care is required.

O-7B-64

Adverse childhood experiences predict adult risk factors for age-related disease: depression, inflammation, and clustering of metabolic risk markers

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Objective: In order to understand why children exposed to adverse psychosocial experiences are at elevated risk for age-related disease, such as cardiovascular disease, we tested whether adverse childhood experiences predict enduring abnormalities in stress-sensitive biological systems–namely the nervous, immune, and endocrine/metabolic systems.
Methods: A representative birth cohort of 1,037 individuals from the Dunedin Multidisciplinary Health and Development Study, based in Dunedin, New Zealand, was followed up from birth to age 32 years in a prospective-longitudinal study (1-3). Appropriate institutional ethics committee clearance and participants' informed consent were obtained. During their first decade of life, Study members were assessed for exposure to 3 adverse psychosocial experiences: socioeconomic disadvantage, maltreatment, and social isolation. At age 32 years, Study members were assessed for the presence of 3 age-related-disease risks: major depression, high inflammation levels (C-reactive protein > 3 mg/L), and clustering of metabolic risk biomarkers (overweight, high blood pressure, high total cholesterol, low HDL, high HbA1c, low VO_2max).
Results: Children exposed to adverse psychosocial experiences were at elevated risk of depression, high inflammation levels, and clustering of metabolic risk markers. Children who had experienced socioeconomic disadvantage (IRR = 1.89; 95% CI = 1.36–2.62), maltreatment (IRR = 1.81; 95% CI = 1.38–2.38), or social isolation (IRR = 1.87; 95% CI = 1.38–2.51) had elevated age-related-disease risks in adulthood. The effects of adverse childhood experiences on age-related-disease risks in adulthood were non-redundant, cumulative, and independent from the influence of established developmental and concurrent risk factors.
Conclusions: Children exposed to adverse psychosocial experiences have enduring emotional, immune, and metabolic abnormalities that contribute explaining their elevated risk for age-related disease. The promotion of healthy psychosocial experiences for children is a necessary, and potentially cost-effective, target for the prevention of age-related disease.

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O-7B-65

Diet during F_0 pregnancy in rats alters weight gain during pregnancy in F_1 and F_2 offspring

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Objective: Pregnancy exerts a substantial energy demand on the mother and reproductive strategies have evolved to facilitate reproductive success when challenged by nutrient scarcity which involve adaptations to the partitioning of energy substrates between mother and fetus[1]. Maternal protein constraint during pregnancy in rats induces an altered phenotype and epigenotype in the F_1 offspring which are transmitted to the F_2 offspring[2,3]. We investigated the effect of feeding F_0 dams diets with different protein and carbohydrate contents on pregnancy outcomes in subsequent generations.

Methods: F_0 Wistar rats were fed standard chow (by weight, 13% protein, 49% carbohydrate, 2.5% fat, 11 MJ/kg) until conception, then either a protein-sufficient (PS; by weight, 17% protein, 63% carbohydrate, 10% fat, 17 MJ/kg ($n = 15$)) or protein-restricted (PR; by weight, 9% protein, 72% carbohydrate, 10% fat, 17MJ/kg ($n = 13$)) diet until term and AIN93G during lactation. Litters were reduced to 8 at birth and offspring were fed AIN93M from weaning. At 70 days, F_1 and F_2 females were mated, and were fed the PS diet during pregnancy and AIN93G during lactation (F_1 PS and PR lineages $n = 15$ each; F_2 PS lineage $n = 13$, PR lineage $n = 12$). Maternal weight and food intake, and litter weight were measured at 7 day intervals. Statistical analysis was by ANOVA with Bonferroni's *post hoc* correction and repeated measures where appropriate (significance differences, $P < 0.05$). Values are mean ± SEM.

Results: There was a significant interaction effect between F_0 diet, generation and gestational age on maternal weight gain. The F_1 and F_2 PS, but not PR, lineage dams gained less weight in pregnancy (F_1 53 ± 9g, F_2 47 ± 7g) than F_0 dams (PS 94 ± 8g, PR 82 ± 9g) due to lower weight gain between d14 and day 21. There was no effect of F_0 diet or generation on maternal food intake during pregnancy and lactation, maternal weight change during lactation, length of gestation, litter size or weight, or weight gain.

Conclusions: Increasing nutrient availability during F_0 pregnancy induced lower weight gain in late gestation in F_1 and F_2 dams. The lack of effect of the PR diet on maternal weight gain suggests an increase in specific nutrients above a threshold level is required. Since there was no effect on litter size or weight, this may represent altered nutrient partitioning in the dams. One possible interpretation is that transition from low (chow) nutrient availability before pregnancy to greater nutrient abundance during pregnancy in the F_0 generation induces in the F_1 and F_2 offspring altered partitioning of energy between deposition or expenditure in the dams, or supply to the offspring during their pregnancies. Thus improving nutrition in the F_0 generation may induce in the F_1 and F_2 offspring an adaptive response which increases nutritional investment in their current pregnancy rather than storing reserves for future litters. This work was supported by the Biotechnology and Biological Sciences Research Council. MAH receives salary support from the British Heart Foundation.

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O-7B-66

Testosterone and male life history: evidence for developmental adaptation in the Philippines

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Objectives: It has been hypothesized that developmental responses to prenatal and early postnatal nutrition, hormones, and other factors may allow the organism to adaptively adjust its biological priorities in anticipation of future conditions. The hypothalamic-pituitary-gonadal axis (HPG) is a prime candidate for adaptive plasticity owing to its perinatal critical period which spans mid-gestation to mid-infancy, and the broad effects that testosterone (T) has on energy allocation and reproductive strategy. Here we test for an effect of early life nutritional stressors on the HPG axis.

Methods: Data come from 756 young adult males (age 20.5–22.5 years) followed longitudinally since their mothers were pregnant with them. We measured salivary waking and evening T and plasma LH in young adulthood, which we relate to bimonthly assessments of infancy weight and infectious morbidity collected when these men were newborns and infants.

Results: We find that infancy stressors (slow weight gain, infectious morbidity) experienced specifically during the perinatal HPG critical period predict reduced waking T in adulthood. These relationships manifest as interactions between early stressors and fatherhood status, suggesting that the degree to which men reduce T in response to fathering is contingent upon early life nutritional cues. Similar relationships are seen for plasma LH, implying a centrally-mediated downregulation of T production rather than peripheral impairment of organ growth.

Conclusions: Our findings provide some of the first evidence for environmentally-induced biological programming of adult testicular function in human males. The implications of these

findings for models of male life history and developmental adaptation will be discussed. Supported by NSF BCS-0542182, Wenner Gren Gr. 7356 and NIH RR20649.

O-7C-67

Salmon in Pregnancy Study (SIPS): Consumption of farmed salmon twice a week during pregnancy increases the EPA and DHA content of maternal and foetal plasma phospholipids

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Long chain n-3 polyunsaturated fatty acids (PUFAs) (i.e. docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3)) exert effects on cell membrane properties, gene expression and eicosanoid metabolism and therefore are biologically important. DHA is an important component of neural and retinal membranes and accumulates rapidly in the brain and retina during the later part of gestation and in early postnatal life. In this way DHA plays a key role in growth, and visual and mental development from very early in life [1]. EPA competes with arachidonic acid for the enzymes responsible for eicosanoid formation and through this action may influence early immune development and play an important role in reducing risk of later inflammatory disorders like allergy [2]. Dietary EPA and DHA are found in oily fish and fish oils. Maternal and foetal EPA and DHA status is likely to be dependent on pre-pregnancy n-3 fatty acid status and maternal intake during pregnancy. Increased requirement for EPA and DHA during pregnancy may be expected because of foetal accretion of these fatty acids[1,3]. There are no studies reporting the effect of increased consumption of oily fish during pregnancy on maternal and foetal EPA and DHA status.

Objective: To determine the effect of increased oily fish consumption during pregnancy on the EPA and DHA content of phospholipids in maternal and cord plasma.

Methods: At 20 weeks gestation, 123 women with high risk of having allergic offspring and low habitual intake of oily fish ($\leqslant$2 times/month) were randomised to consume two portions of salmon per week (each portion provided 2 g of n-3 PUFA) (salmon group) or to continue their habitual diet of low oily fish consumption until delivery (control group). The women attended a clinic in the fasted state at weeks 20 (n = 123) and 32–34 (n = 111) of pregnancy, at which blood samples were collected, and a food frequency questionnaire administered. Umbilical cord blood was collected at birth (n = 101). Plasma was isolated at all timepoints and the phospholipid (PL) fatty acid composition was determined by gas chromatography. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: In the control group EPA and DHA (% of total fatty acids) in maternal plasma PL decreased significantly from week 20 to 34 of pregnancy: EPA from 0.62% to 0.4% ($p < 0.001$), DHA from 4.13% to 3.96% ($p = 0.013$). In the salmon group EPA and DHA in the maternal plasma PL increased significantly from week 20 to 34 of pregnancy: EPA from 0.71% to 0.88% ($p = 0.01$), DHA from 4.77% to 5.10% ($p = 0.001$). Cord plasma PL EPA and DHA contents were significantly higher in the salmon group compared with the control group: EPA 0.63% vs. 0.32% ($p < 0.001$), DHA 7.40% vs. 6.44% ($p = 0.001$).

Conclusions: Eating oily fish twice a week during pregnancy results in a higher maternal status of long chain n-3 fatty acids (both EPA and DHA) and in a higher status of these fatty acids in cord plasma. This may result in improved growth, development and health in the offspring. This work is supported by the European Commission (Framework 6) and forms part of the AquaMax integrated project (FOOD-CT-2006-016249-2). The authors thank staff at the Princess Anne Hospital, Southampton and the Medical Research Council Epidemiology Resource Centre.

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O-7C-68

Birth weight, childhood respiratory illness and lung function at ages 14 and 50 years: The Newcastle Thousand Families Study

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Although low birth weight and childhood respiratory tract infections have both been associated with reduced adult lung function, little is known about the timing of these associations during life. We have previously shown low birth weight and more frequent childhood lower respiratory tract infections to be associated with lower forced expiratory volume in the first second (FEV_1) at age 49–51 in the Newcastle Thousand Families Study[1].

Objective: The objective of this study was to examine how these, and other, factors influenced FEV_1 at age 14 years and between ages 14 and 49–51 years.

Methods: The Newcastle Thousand Families Study is a prospective study that began in 1947 when all 1142 children born to mothers resident in the city of Newcastle upon Tyne in northern England were recruited. Detailed information on many aspects of their lives was collected prospectively during childhood, including early growth, infections and socio-economic conditions. Birth weight was standardised for sex and gestational age. The number of episodes of lower

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

respiratory tract infections, colds and other upper respiratory tract infections were recorded as counts, while history of other infections, such as measles, whooping cough, tuberculosis and influenza in the first five years were coded as dichotomous (ever had or not) variables. At age 14 years, 252 members of the cohort were recruited into a nested case-control study of respiratory health, which included measurement of FEV_1. 122 of these were measured again at aged 49-51 when both questionnaires and a clinical assessment were used to collect a range of health (including FEV_1 measured by spirometry), lifestyle and socio-economic information. Linear regression models were used to examine cross-sectional and longitudinal influences on FEV_1. The total influence of each variable was determined using minimally adjusted models including the test variable and, in the case of the percentage change, FEV_1 at baseline. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Lower current height ($p < 0.001$), lower current BMI ($p < 0.001$), being breast fed for less than four weeks ($p = 0.028$), childhood history of severe respiratory illness (i.e. case status in the nested case-control study) ($p = 0.014$), childhood history of asthma ($p = 0.004$), childhood history of tuberculosis ($p = 0.023$), and birth into a lower social class ($p = 0.049$) were all significant independent predictors of lower FEV_1 at age 14 years. Correspondingly, being female ($p < 0.001$), higher FEV_1 at age 14 years ($p < 0.001$), lower standardised birth weight ($p = 0.025$), greater lifetime cigarettes smoked ($p = 0.007$), and childhood history of severe respiratory illness ($p = 0.047$) were all independently associated with a greater decline (or a smaller increase) in FEV_1 between ages 14 and 49–51 years. Conclusion: This study suggests that the change in FEV_1 between youth and middle age depends on several factors acting throughout life. Higher FEV_1 at 14, being female, more cigarettes smoked, severe childhood respiratory illness, and lower standardised birth weight were all predictive of a greater decline (or smaller increase) in FEV_1. Support: This analysis was funded by the Newcastle Healthcare Charity, UK.

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O-7C-69

Fetal and infant growth patterns are associated with the development of atopy and wheezing disorders in early childhood

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Objective: To determine whether fetal and early infant growth patterns influence the risk of developing wheeze or atopy in early childhood.

Methods: Data on wheeze at ages 6, 12, 24 and 36 months were collected from 1548 term offspring of Southampton Women's Survey participants. Atopic status was determined by skin prick testing at ages 1 and 3 years. Detailed respiratory phenotyping at age 6 years is ongoing; to date preliminary data is available for 469 children. Growth was measured using serial antenatal ultrasound scans and anthropometry during infancy. We used Poisson regression to derive relative risks for outcomes in relation to conditional measurements of growth velocity. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Atopy risk at age 3 years increased by 46% per SD increase in abdominal circumference growth from 11–19 weeks' gestation ($p = 0.007$). Conversely, each SD decrease in abdominal growth from 19–34 weeks' gestation increased atopy risk by 20% ($p = 0.011$). Atopic wheeze risk at age 3 years decreased by 20% per SD increase in 19–34 week abdominal growth ($p = 0.046$); greater increases in weight and adiposity in the first year of life were also associated with atopic wheeze. Non-atopic wheeze risk increased by 8% per SD decrease in crown-rump length at 11 weeks' gestation, and by 6% per SD increase in adiposity during the first 6 months of infancy ($p = 0.024$). Preliminary analyses of follow up data at age 6 years suggest persistence of the association of abdominal circumference growth faltering with atopic wheeze risk, and showed that both transient and persistent wheeze were significantly positively associated with adiposity gain between birth and 6 months.

Conclusions: The association of rapid early growth followed by growth faltering with later atopy suggests that immune development is sensitive to impaired fetal nutrient supply. Small airways may underlie the association between smaller fetal size and non-atopic wheeze. The association between postnatal adiposity gain and wheezing disorders may reflect consequences of impaired fetal growth, although rapid postnatal weight gain may itself be harmful.

O-7C-70

Developmental origins of lung function at 6 years of age: does the intrauterine amniotic fluid volume matter?

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Objective: The evaluation of amniotic fluid volume (AFV) is an essential component of fetal examination with ultrasound in pregnancy. Abnormal AVF in high risk pregnancy has been linked with adverse pregnancy outcomes. The long term

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

effects of abnormal AFV on infant and childhood pulmonary function remains unknown. This investigation examined the relationships between AFV in unselected obstetric population of women and the subsequent respiratory function in the children in early childhood.

Methods: This study evaluated children whose mothers had participated in a randomised controlled trial evaluating effects of multiple antenatal ultrasounds on pregnancy outcomes (The Raine Study[1], 1989–1992). Women randomised to the ultrasound intensive group underwent a protocol of 5 ultrasound scans from 18 pregnancy weeks. The amniotic fluid index was measured during each ultrasound, and gestational age specific cut-offs[2] were used to determine if AFV was normal (N), oligohydramnios (O) and polyhydramnios (P). Primary respiratory outcomes included wheezing and non-wheezing lower respiratory infections (LRI) at ages 1, 2, 3 and 6 years; current asthma, wheezing and non-wheezing LRI phenotypes (nil, transient up to age 3, late onset at age 6, persistent) and lung function[3] (forced vital capacity, FVC; forced expired volume, FEV1; forced expiratory flow between 25%–75% of vital capacity, $FEF_{25–75}$) at age 6. Appropriate institutional ethics committee clearances and participants' informed consent were obtained for all studies.

Results: There were 5895 AFV assessments obtained on 1276 singleton pregnancies (median 4, range 1–7). The AFV was consistently within the normal range in 479 (37.5%); N-O combination in 602 (47.2%); N-P combination in 135 (10.6%); and N-P-O combination in 60 (4.7%) pregnancies. Persistent oligohydramnios or polyhydramios was only observed in 4 pregnancies. The incidence of polyhydramnios decreased with advancing gestation (12.7% to 1.2%). The delivery of a small for gestational age infant was associated with less frequent normal AFV and an increased N-O combination ($p = 0.002$). No effects of AFV on wheezing LRI at any age or asthma at age 6 were evident. N-O-P combination was associated with an increased likelihood of non-wheezing LRI at age 6 (25% vs 10.7%, adjusted OR = 2.97 95%CI 1.48–5.97, $p = 0.002$), and with an increased likelihood of persistent non-wheezing LRI phenotype (12.5% vs 3.6%, adjusted OR = 3.76 95%CI 1.47–9.63, $p = 0.006$). Sporadic presence of increased volume or polyhydramnios was associated with an increase in $FEF_{25–75}$ (adjusted $\beta = 0.098$ 95%CI 0.013–0.183, $p = 0.024$). No effects on FVC and FEV1 were evident.

Conclusions: Amniotic fluid volumes in unselected pregnancies are not associated with the development in early childhood of asthma or wheezing lower respiratory infections. Pregnancies with wide variations in volume described as changing from too much to too little to normal are associated with an increased risk of late onset and persistent non-wheezing lower respiratory infections. Within the spectrum of normal volumes, increases in amniotic fluid volume in the second pregnancy trimester may result in improved efficiency in the small airways.

1. J.P. Newnham *et al.*, *Lancet*, 342: 887–891, 1993.
2. E.F. Magann *et al.*, *Am J Obstet Gynecol.*, 182:1581–1588, 2000.
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O-8A-71

Male obesity: consequences for embryo development and fetal outcomes

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Objective: Understanding how maternal health and body weight influence the ability to conceive, subsequent pregnancy development and health of the offspring, has been at the centre of DoHAD research endeavours for many decades. Maternal obesity correlates with a decrease in the rate of conception, fertilisation rate in vitro, reduced embryo grade and a range of reproductive complications. In contrast, understanding how similar paternal aetiology's influence embryo health, and the capacity to develop and form a pregnancy have essentially been ignored. The aim of this study was to use a rodent model of male diet-induced obesity, to determine whether resultant embryo development is compromised and whether fetal and placental growth is adversely affected.

Methods: Thirty-six, 6 week old C57BL/6 male mice were fed a standard rodent chow (C) (AIN93M) or a high fat diet (HFD) (SF00-219, Specialty Feeds, Australia) providing 22% fat, a Western style diet. Body weight was measured weekly and after a period of 8 weeks, males were mated with superovulated CBAxC57BL/6 females and zygotes collected for culture to the blastocyst stage, in G1.2/G2.2 series media. Embryo development was assessed daily over a period of 92 hr, then a) cell number determined for day 5 blastocysts using a differential staining protocol, or b) embryos transferred into pseudopregnant recipient mice ($n = 10$ embryos per female), to examine fetal and placental outcomes at postmortem on day 18 of pregnancy. Male mice were euthenased and blood samples collected for plasma metabolite determination using a COBAS Mira analyzer, and adipose depots dissected and weighed. Differences between treatments were assessed using Univariate GLM or chi-square analysis. Appropriate institutional ethics committee clearance was obtained.

Results: At the end of the 8 week feeding period, males from the two groups were significantly different, with average body weight (30.5 g vs 26.8 g, $p < 0.001$) and fat weight (1116 mg vs 530 mg, $p = 0.01$ combined peritoneal and retroperitoneal depots) higher for males fed the HFD. Similarly, plasma FFA (0.98 vs 0.78 meq/L, $p = 0.008$) and cholesterol (4.69 vs

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

2.79 mmol/L, $p < 0.001$) were also significantly higher. Embryo development was markedly perturbed at all stages assessed, including reduced cleavage on day 2 (60.3% vs 84.3%, $p < 0.001$) and development to blastocyst on day 4 of culture (29.5% vs 58.5%, $p < 0.001$) relative to control sired embryos. Embryos from HFD males implanted at a reduced rate (86.7% vs 72.5%, $p < 0.03$) and the number of fetuses was reduced (22.5% vs 38.7% fetuses, $p < 0.03$), although fetal and placental weight was not different between dietary groups. A small number of pregnancies went to term and preliminary data indicate that offspring weight at weaning was significantly heavier when pups were born to HFD males ($p < 0.05$).

Conclusions: This study demonstrates in a mouse model that paternal HFD exposure prior to conception does not only affect events associated with the sperms ability to fertilise the egg but also impairs pre-implantation embryo development and the health of an ensuing pregnancy. This highlights the important contribution that paternal health around the time of conception has for a pregnancy and indicates that health messages aimed at prospective fathers could be beneficial.

O-8A-72

Epigenetic marks at birth predict childhood body composition at age 9 years

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Objective: While genomic variation explains only a modest proportion of the risk of cardiovascular and metabolic disease, animal models demonstrate that maternal environmental influences alter epigenetic processes in the offspring, with important effects on their later body composition and cardio-metabolic function. Whether such processes operate in humans has not been examined.

Methods: Using DNA extracted from stored umbilical cord tissue from healthy neonates who were subsequently extensively phenotyped, we related the DNA methylation status of specific CpGs 5' from candidate genes to body composition measured by DXA scanning at age 9 years. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Methylation, measured by the Sequenom MassARRAY system, varied greatly at particular CpG sites. Of 68 CpGs studied, 31 had a median methylation $\geq 5\%$ and a 5-95% range $\geq 10\%$. Independently of sex, there were strong correlations of the degree of methylation in specific CpGs in eNOS with childhood fat mass (Pearson correlation $r_p = 0.42$, $n = 66$) and trunk/limb fat ratio ($r_p = 0.33$, $n = 66$), and in RXRA methylation with fat mass and percentage fat mass ($r_p = 0.32$ and 0.29, respectively, $n = 64$) (all $p < 0.005$, taking account of methylation at other CpG sites). Methylation at these sites was not linked to birth weight. Controlling for sex, perinatal epigenetic marks explained more than 40% of the variance in body composition at age 9 years.

Conclusions: Epigenetic marks at birth predict a significant proportion of the variance in later childhood adiposity, indicating that substantial components of later metabolic disease risk are induced before birth. Perinatal epigenetic analysis may have utility in identifying individual vulnerability to later chronic non-communicable disease. MAH and JSJ are supported by the British Heart Foundation. PDG and AS are funded by the National Research Centre for Growth and Development (New Zealand). BSE is supported by the Agency for Science, Technology and Research (Singapore). This work was supported by the charity WellChild (previously Children Nationwide), by the University of Southampton, by the UK Medical Research Council and the EpiGen consortium.

O-8A-73

Characterization of the mechanisms of epigenetic reprogramming in the early embryo

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Objective: DOHaD shows that epigenetic perturbation during development can cause whole-of-life homeostatic changes. The most profound epigenetic reprogramming of the organism's life occurs during the preimplantation stage of development. The current paradigm implicates active global DNA demethylation of the paternal pronucleus soon after fertilization, but passive demethylation of the maternally-derived genome over many subsequent cell-cycles. This global demethylation is followed is followed by selective remethylation commencing at around the blastocyst stage. The apparent asymmetry in reprogramming of the maternally- and paternally-derived genomes has been difficult to reconcile with other biological processes prompting us to re-examine this evidence.

Methods: DNA methylation levels were examined in mouse zygotes by immunolocalization using methylcytosine specific antibodies. Zygotes were isolated from the oviduct at times after hCG and staged for pronuclei maturity (PN1-5, least to most mature) or metaphase commencement.

Results: We found DNA methylation levels to be high in PN1 – 2 stages, but then progressively declined. By PN5 stage, staining was barely evident and extensive demethylation was evident in both the male and female pronuclei. We found no methylcytosine staining in any metaphase chromosomes at syngamy. This

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

contrasts with current evidence for a marked asymmetry in demethylation of the maternal and paternal pronuclei. Most past studies used zygotes that had been collected after fertilization and then cultured in vitro, or produced by IVF. When we prepared zygotes by these methods we found that demethylation was delayed, and that this particularly affected the female pronucleus. When zygotes were generated by IVF this asynchrony was further exacerbated. Many cultured post-syngamal zygotes showed extensive methyl-cytosine staining of the metaphase chromosomes. This new finding creates a paradox – if global demethylation normally occurs by the time of syngamy, yet remethylation does not occur until the blastocyst stage, how can cleavage stage embryos show DNA methylation? We found that two-cell stage embryos collected direct from the oviduct showed high levels of cytosine methylation. We assessed whether this accumulation of cytosine methylation from the time of syngamy to the early 2-cell stage was a consequence of DNA methyltransferase activity by treating late stage zygotes with the DNMT inhibitor, RG108 (5 μM), for the period of development spanning PN 5 to the early two-cell stage. RG108 treatment significantly reduced the remethylation ($p < 0.001$) that occurs in two-cell embryos, and also reduced the developmental potential of the treated embryos ($p < 0.01$).

Conclusions: Our results show for the first time that global demethylation of the genome is complete by the time of syngamy and that de novo re-methylation of the genome occurs as early as the 2-cell stage of development. The results substantially change our understanding of epigenetic reprogramming in the early embryo and show the zygote to two-cell transition to be profoundly more epigenetically active then previously thought. Treatments as simple as embryo culture can perturb these reprogramming events indicating a hitherto unexpected epigenetic fragility of the early embryo.

O-8B-74

Maternal stress during pregnancy and pediatric diseases: a population-based cohort study

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Objective: Maternal stress during pregnancy may increase the offspring's risk for several diseases.

Methods: We assessed in a national birth cohort with prospective data the association between maternal stress during pregnancy and offspring diseases during childhood (ICD-10 diagnoses from national registries; N = 66203). We used two a priori defined indicators of common forms of stress, life stress (perceived burdens in major areas of life) and emotional stress. We conducted Cox or logistic regression analyses, controlling for maternal stress after pregnancy and potential confounders, for each diagnostic category. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Median age at end of follow-up was 6.2 (range: 3.6–8.9) years. Life stress (highest compared to lowest quartile) was associated with an increased risk of conditions originating in the perinatal period [odds ratio (OR) = 1.13; 95% confidence interval (CI) = 1.06–1.21], malformations (OR = 1.17; CI = 1.06–1.28), and the first onset of infections [hazard ratio (HR) = 1.28; CI = 1.17–1.39], mental disorders (age 0–2.5 years: HR = 2.03; CI = 1.32–3.14), eye (age 0–4.5 years: HR = 1.27; CI = 1.06–1.53), ear (HR = 1.36; CI = 1.23–1.51), respiratory (HR = 1.27; CI = 1.19–1.35), digestive (HR = 1.23; CI = 1.11–1.37), skin (HR = 1.24; CI = 1.09–1.43), musculoskeletal (HR = 1.15; CI = 1.01–1.30), and genitourinary diseases (HR = 1.25; CI = 1.08–1.45). Emotional stress was associated with an increased risk for the first onset of infections (HR = 1.09; CI = 1.01–1.18) and a decreased risk for the first onset of endocrine (HR = 0.81; CI = 0.67–0.99), eye (HR = 0.84; CI = 0.71–0.99), and circulatory diseases (age 0-3 years: HR = 0.63; CI = 0.42–0.95).

Conclusions: Maternal life stress during pregnancy may be an early risk factor for impaired child health. This is the first comprehensive study on maternal stress during pregnancy and a wide spectrum of diseases during childhood. If some of these findings are causal, the results may open new approaches to reduce childhood diseases. Support source: The Danish National Research Foundation has established the Danish Epidemiology Science Centre that initiated and created the Danish National Birth Cohort. The cohort is furthermore a result of a major grant from this Foundation. Additional support for the Danish National Birth Cohort is obtained from the Pharmacy Foundation, the Egmont Foundation, the March of Dimes Birth Defects Foundation, the Augustinus Foundation, and the Health Foundation. Support: German National Academic Foundation (PhD scholarship to MT), and the Swiss National Science Foundation (SNSF), project no.51A240–104890, (to GM).

O-8B-75

Elevated stress and low social support contribute to poor birth outcomes by altering maternal inflammatory cytokine production

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https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

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Objective: Increased inflammation during pregnancy is associated with increases in premature labor and pregnancy complications. Our previous work showed that stress and social support affect inflammatory cytokines in a manner which may endanger human pregnancy, although we did not directly make these connections. The present study directly addressed this by assessing relationships between stress, pregnancy-specific distress and support, circulating cytokines, and birth outcomes.
Methods: A sample of 130 pregnant women (70% Latina, 30% non-Latina) was recruited though Denver Health Medical Center. Subjects provided informed consent and then completed the Denver Maternal Health Assessment, the Revised Pregnancy Distress Questionnaire, and the Prenatal Social Support Instrument and provided blood samples at 12–19 and 35–40 weeks of pregnancy. Serum levels as well as stimulated in vitro production of TNF-α, IL-6 and IL-10 were determined in duplicate via ELISA (R & D Systems). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Data regarding birth outcomes were gathered through chart extraction. Stress, distress, and support data were examined in relationship to the cytokine measures across pregnancy, and levels of cytokines were examined regarding their predictive value for birth outcomes. Elevated IL-6 and TNF-α were associated with lower levels of support and higher stress throughout pregnancy ($p < .0445$ and $p < .01$, respectively), and higher social support was associated with higher IL-10 late in pregnancy ($p < .031$). TNF-a was related to higher pregnancy-specific distress early in gestation ($p < .001$). Elevated TNF-a and lower social support late in pregnancy predicted lower 1-minute APGAR scores, lower gestational age at birth, and increased occurrence of birth complications including meconium aspiration, nuchal cord, and asphyxia (p values $<.05$ in all cases).
Conclusions: Together, these data suggest that increased maternal stress and pregnancy-specific distress as well as lower social support during pregnancy are related to increases in both the production of inflammatory mediators by immune cells and circulating levels of these mediators and that these changes are related to an increased risk of untoward birth outcomes. Ongoing and future work is addressing the degree to which changes in psychosocial factors and inflammatory cytokines are useful as predictors of clinical outcome as well as the usefulness of stress-management interventions in preventing stress-related poor birth outcomes.

O-8B-76

Can digit-length ratios be used as a marker of prenatal undernutrition? Evidence from the Dutch Hunger Winter Families Study

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Objective: Digit lengths, and in particular the ratio of the 2nd to 4th digit (2D4D), have been linked to many psychosocial characteristics thought to have developmental origins. We examined in a large cohort whether exposure to undernutrition during gestation was associated with the lengths of the second (2D) and fourth (4D) digits or the 2D4D.
Methods: We studied men and women (1) born in one of three maternity centers in western Netherlands whose mothers were exposed to a limited period of famine immediately prior to or during the pregnancy ($n = 427$); (2) born in the same hospitals to mothers not exposed to famine during the pregnancy ($n = 224$) or same-sex siblings of individuals in groups 1 and 2 ($n = 312$). We measured 2D and 4D on both hands using calipers. We computed 2D4D as 2D/4D. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Mean 2D and 4D lengths were 73.4 (SD 5.2) and 74.9 (5.5) mm respectively, and did not differ by hand. The 2D4D ratio was 0.981 (SD 0.031). Correlations between left and right hands within individuals were 0.95 for 2D and 4D and 0.61 for 2D4D; same-hand correlations within sibling pairs were 0.64 for 2D, 0.61 for 4D and 0.27 for 2D4D (all $p < 0.001$). 2D and 4D were associated with male gender and height (all $p < 0.001$), and weakly with body mass index (BMI). 2D4D was 0.0085 (95% CI 0.0029, 0.0141) lower among males, and was not associated with height (sex-adjusted; 0.0002 per cm; 95% −0.0001, 0.0006) or BMI (sex- and height-adjusted; 0.0007 per kg/m^2; 95% CI −0.0003, 0.0011). Exposure to famine during weeks 11–20 of gestation was associated with an increase in digit lengths (0.0085 (95% CI 0.0017, 0.0151; $p = 0.014$) for 2D and 0.0067 (95% CI −0.0008, 0.0143; $p = 0.08$) for 4D. No other period of famine exposure was associated with either digit length. Exposure to famine prior to or during gestation was not associated with 2D4D.
Conclusions: The minor increment in 2D and 4D associated with famine exposure in gestational weeks 11–20 may point to early growth determinants of interest. The 2D4D ratio was not related to prenatal exposure to famine and therefore is unlikely to serve as an offspring marker for generalized prenatal undernutrition.

O-8C-77

Effect of vitamin D deficiency during pregnancy on offspring bone structure and quality

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https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

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Objective: Maternal adaptations during pregnancy and lactation are thought to provide the developing fetus with the required calcium independently of maternal vitamin D (VitD) levels, and only after birth, that dependency on VitD appears necessary, at least with respect to calcium metabolism and skeletal health. However, Javaid *et al.*[1] found a significant correlation between reduced maternal VitD concentrations and reduced calcium transfer during pregnancy and decreased bone mineral density and bone mineral content in related offspring at nine years of age. We used a rat model to determine if maternal VitD deficiency during pregnancy had a deleterious effect on the offspring at birth, and whether any differences could be reversed by the reintroduction of VitD.
Methods: Rat dams were housed under UV-free conditions to eliminate light as a source of VitD. From 4 weeks of age, dams were fed a VitD deplete diet, mated at 10 weeks of age, and maintained on the deplete diet until delivery and then switched to the control diet. Offspring received the control diet from weaning. Offspring femur, tibia, and vertebra were analysed over a range of timepoints.
Results: At birth (n = 12 for each sex/diet group), male offspring from the VitD deplete group had less connected trabecular structure in the femur together with reduced trabecular thickness (all $p = 0.05$). The tibia also showed a less connected trabecular structure ($p = 0.006$). Whereas the vertebra showed reduced trabecular thickness, with a more connected trabecular structure ($p = 0.003$ and 0.04). Female offspring in the deplete group were not affected. By 21 days of age (n = 12 for each sex/diet group), the male deplete group offspring showed no differences compared to controls. In contrast, the female offspring showed femural differences with increased bone volume and a more rod-like trabecular structure ($p = 0.04$ and 0.02). At 70 days of age (n = 6 for each sex/diet group), the female deplete group showed increased midshaft wall thickness of the femur and tibia ($p = 0.004$ and 0.02), increased tibial midshaft load at failure ($p = 0.05$), and a more connected trabecular structure at the distal tibia ($p = 0.04$). Male offspring were unaffected. No structural differences were found in either male or female offspring in the VitD deplete groups at 140 days of age (n = 6 for each sex/diet group). However, there was evidence of premature mineralisation of secondary ossification centres in both these groups compared to controls.
Conclusions: Male offspring showed differences in bone structure, due to VitD depletion during pregnancy, earlier than female offspring. However, these differences were resolved quicker in males than in females. Both sexes showed indications of premature bone growth senescence. Although maternal VitD levels are believed not to affect calcium transport and skeletal health in the growing fetus, we present evidence this is not the case. Transient prenatal VitD depletion altering the trajectory of bone development is associated with persistent changes in the offspring. Hypovitaminosis D during pregnancy ranges from 5–85% depending on study group; hence the bone alterations shown here could have major health consequences impact in later life.

1. M.K. Javaid *et al.*, *Lancet*, 367:36-43, 2006.

O-8C-78

Fetal bone growth in pregnant adolescents is associated with maternal vitamin D status

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Objective: Longitudinal changes in maternal bone mass, fetal bone growth and calcitropic hormones across gestation will be assessed in a group of 200 pregnant adolescents (≤18 y of age). Relationships between maternal and neonatal calcitropic hormones and placental calcium transporters will be explored.
Methods: Maternal bone mass (by heel ultrasound) and fetal femur (FL) and humerus (HL) length (by sonogram) were assessed at study entry (~12 wks gestation), mid-gestation (~24 wks gestation) and late pregnancy (~34 wks gestation). Maternal blood collected at mid-gestation and delivery and cord blood at birth were analysed for 25(OH)D, $1,25(OH)_2D$, intact PTH, IGF-1, osteocalcin, serum N-telopeptide (NTX), serum osteoprotegerin (OPG) and leptin. Dietary 24-h recalls and FFQ's were obtained at each visit. Placentas were weighed at delivery and samples obtained to assess expression of placental calcium transporters. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: A group of 164 teens (66% black/34% white/24% Hispanic) 17.0 ± 1.1 y at entry (range 13.6–18.7 y) have been recruited. Teens delivered at 39.1 ± 2.8 wks gestation (27.4–41.9 wks) and gained 17.0 ± 7.2 kg over the course of pregnancy (4.5–36.4 kg). Infant birth weight was 3129 ± 643 g (n = 83; 1052–4705 g); 10% of infants were LBW (<2500 g) and preterm birth (<37 wks) occurred in 10% of teens. Calcium intake averaged 1033 ± 483 mg/d (209–2636 mg/d) and was not associated with gestational age at delivery or fetal bone growth. Placental weight averaged 589 ± 152 g (n = 88, range 271–979 g) and did not significantly differ as a function of age or race but was positively correlated with birth weight ($p < 0.0001$, n = 65), maternal weight gain ($p = 0.03$) and calcium intake ($p < 0.03$). PTH increased over gestation (27.2 ± 7.5; n = 27 vs. 40.7 ± 17 pg/mL; n = 26) and by delivery was elevated (>46 pg/mL) in 46% of teens. Dietary Ca intake was not significantly associated with PTH at delivery or net weight gain over pregnancy. Vitamin D status at

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

mid-gestation averaged 23.3 ± 12.4 ng/mL (n = 127) and 22.5 ± 10.7 (n = 80) in neonates and D insufficiency (≤20 ng/mL) was evident in 46% of teens and 51% of their neonates. Maternal 25(OH)D at delivery was significantly correlated with rate of fetal femur growth (p = 0.05, n = 83) and rate of fetal humerus growth per week (p = 0.008, n = 63). Placental weight was correlated with change in FL (p = 0.03, n = 65) and HL per week (p = 0.01, n = 45). Fetal bone growth was not significantly associated with osteocalcin, Ca intake or NTX. An inverse relationship between maternal age and FL approached significance (p < = 0.06, n = 84).

Conclusions: Maternal D insufficiency was prevalent among pregnant adolescents and their newborns and PTH was elevated in nearly 50% of this cohort at parturition. Maternal 25(OH)D status was associated with a decrease in fetal long bone growth. Long term implications of these findings merit additional attention. This project was supported by National Research Initiative Grant 2005-35200 from the USDA Cooperative State Research, Education and Extension Service.

O-8C-79

Birth weight and bone mass in early adulthood: results from a birth cohort study in Ribeirão Preto, Brazil

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Objective: To investigate the relationship between birth weight and adult bone mass.

Methods: Data derived from a birth cohort and comprised 247 men and 266 women aged 23–24 years born and still resident in Ribeirão Preto, Brazil. Subjects underwent bone densitometry by dual-energy X-ray absorptiometry. Bone area, bone mineral content (BMC) and bone mineral density (BMD) were estimated at lumbar spine, femoral neck and total proximal femur. Pearson's correlation coefficients were employed. Trends in mean differences between groups of birth weight tertiles were explored by linear regression and three multiple linear regression models were performed: (1) adjusted for sex; (2) adjusted for sex and adult weight; and (3) adjusted for sex, adult weight, physical activity, smoking habit, calcium intake, and alcohol consumption. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Birth weight was positively associated with bone area of lumbar spine, femoral neck and proximal femur; both for men (r = 0.22, 0.17 and 0.22, $p \leq 0.01$; respectively) and women (r = 0.19, 0.16, and 0.16, $p \leq 0.05$; respectively). Men in the highest tertile of birth weight had greater BMC at all three studied bone sites than their counterparts in the lowest thirds (p for trend <0.01). For women, this trend was observed only at lumbar spine ($p = 0.014$).

			Birth weight (kg)			
			(< 3,108)	(3,108 – 3,500)	(> 3,500)	
	Site	Measure	n = 81	n = 83	n = 83	p for trend
Men	Lumbar spine	Area (cm^2)	64.94	66.84	68.52	< 0.001
		BMC (g)	67.68	71.33	74.29	0.001
		BMD (g/cm^2)	1.04	1.06	1.08	0.034
	Femoral neck	Area (cm^2)	5.17	5.30	5.38	< 0.001
		BMC (g)	5.12	5.25	5.50	0.012
		BMD (g/cm^2)	0.99	0.99	1.02	0.208
	Proximal femur	Area (cm^2)	37.62	39.03	39.79	< 0.001
		BMC (g)	40.67	42.14	44.31	0.005
		BMD (g/cm^2)	1.08	1.08	1.11	0.237
			Birth weight (kg)			
			(<3,000)	(3,000–3,372)	(> 3,372)	
			n = 93	n = 83	n = 90	
Women	Lumbar spine	Area (cm^2)	54.28	54.74	56.66	0.003
		BMC (g)	52.89	54.48	56.63	0.004
		BMD (g/cm^2)	0.97	0.99	1.00	0.087
	Femoral neck	Area (cm^2)	4.50	4.52	4.65	0.012
		BMC (g)	3.73	3.77	3.87	0.083
		BMD (g/cm^2)	0.83	0.84	0.83	0.749
	Proximal femur	Area (cm^2)	28.77	28.83	29.88	0.007
		BMC (g)	25.73	25.94	26.92	0.055
		BMD (g/cm^2)	0.89	0.90	0.90	0.627

Birth weight retained the positive associations with BMC and bone area, at all sites, after adjustments for adult weight and lifestyle factors ($p < 0.05$). Model 3 explained 50% of the lumbar spine, 63% of femoral neck and 73% of proximal femur BMC variation.

Conclusions: According to literature[1], our data support birth weight as a determinant of bone mass, in a sample of young adults from Brazil. The independent effect of birth weight was restricted to BMC and bone area. Optimizing fetal nutrition might be strategic for early prevention of osteoporosis. Coordination Support of Higher Education (CAPES), National Council for Scientific and Technological Development (CNPQ), Foundation for Research Support of the State of São Paulo (FAPESP).

1. C. Cooper *et al.*, *Osteoporosis Int.*, 17:337–347, 2006.

O-9A-80

Developmental programming of cardiovascular disease: The roles of prenatal hypoxia and oxidative stress

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https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

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Objective: We put forward the hypothesis that oxidative stress in the fetal cardiovascular system underlies the molecular basis via which prenatal hypoxia contributes to the developmental programming of cardiovascular disease. If true, treatment with antioxidants of pregnancies complicated with reduced oxygen delivery to the fetus, such as during pre-eclampsia or placental insufficiency should diminish the hypoxia-induced origins of cardiovascular disease. The work not only offers insight into mechanisms underlying the association between poor intrauterine conditions and increased risk of heart disease in adulthood, but also possible clinical intervention. Using an integrative approach at the isolated organ, cellular and molecular levels, we investigated the longitudinal effects on the cardiovascular system of the offspring at the end of gestation and adulthood of maternal treatment with melatonin during hypoxic pregnancy in the rat. Melatonin is widely recognised as a potent antioxidant and one of the few hormones that crosses the placenta unaltered.

Methods: On pregnancy day 15, 84 Wistar rats (n = 14 per group) were divided into control (21% O_2), hypoxic (10% O_2) or undernourished (35% reduction in food intake) pregnancy, with and without maternal melatonin (5 $\mu g\,ml^{-1}$ drinking water). Undernourished groups served as pair-fed controls for the hypoxia-induced reductions in maternal food intake. On day 20, half of the dams were anaesthetised, the pups and placentae dissected, weighed and fixed or frozen. The remaining dams were allowed to deliver. At birth, litters were culled to 8 (4 males, 4 females) and mother and pups maintained in normoxia. Following weaning, offspring were maintained until adulthood. At 4 months, following euthanasia, organs were fixed or frozen. Fixed tissue was used for histology. Pro- and anti-oxidant protein expression was determined by Western blot. Mesenteric vessels from adults were isolated for wire myography. To control of sex and within litter variation, 1–2 male offspring from any litter for any variable measured were taken. Results: Relative to controls, the reduction in birth weight (H: $-9 \pm 1\%$, U: $-11 \pm 1\%$, mean ± S.E.M, $P < 0.05$) and catch up growth to four months (fractional growth: H: $+8 \pm 2\%$, U: $+11 \pm 2\%$, $P < 0.05$) were similar in offspring from hypoxic and undernourished pregnancy. Maternal melatonin significantly increased the placental expression of catalase and MnSOD and improved birth weight in undernourished (UM: $-4 \pm 1\%$, $P < 0.05$) but not hypoxic (HM: $-9 + 1\%$) pregnancy. Hypoxic but not undernourished pregnancy resulted in thickening of the fetal aortic wall (Fig. 1A) with no morphological alterations to the fetal heart. In marked contrast, by adulthood, offspring of hypoxic but not undernourished pregnancy showed dilated cardiomyopathy with increased mesenteric vasoconstrictor reactivity to PE and U46619 (Fig. 1B&C), but no changes in aortic structure. Maternal melatonin relieved all of the adverse cardiovascular consequences in fetal and adult offspring of hypoxic pregnancy.

Conclusions: Developmental hypoxia has differential effects on the cardiovascular phenotype of fetal and adult offspring, but maternal treatment with melatonin protects against both. The data support that oxidative stress in the fetal cardiovascular system underlies the molecular basis via which prenatal hypoxia contributes to the developmental programming of cardiovascular disease. Support: The British Heart Foundation, BBSRC, The Royal Society.

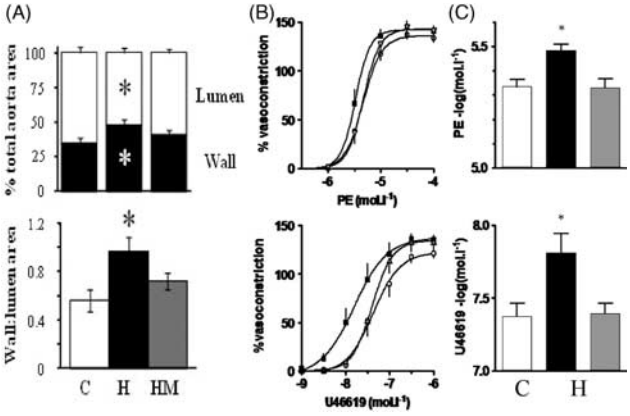

Figure 1. Mean ± SEM for: (A) areas of the fetal aorta either as a % of the total vessel area or the wall: lumen area ratio; (B) the concentration response curves; and (C) the sensitivity (PD2) in response to PE or U46619 in mesenteric arteries from 4-month-old adults. (C, control or circles; H, hypoxic or squares; HM, hypoxia + melatonin or triangles; n=7 per group). *$P < 0.05$ vs C ANOVA + Tukey test.

O-9A-81

Mechanisms of endothelium-dependent vasodilation in young and aged offspring born growth restricted

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Objective: Numerous epidemiological studies have shown that cardiovascular dysfunction in adult life may be programmed by compromised growth *in utero*. Cardiovascular diseases are the leading cause of death in the world today and, in addition, the incidence of cardiovascular disease rises dramatically after 60 years of age in both males and females. Aging is a risk factor for vascular endothelial-dependent dysfunction. The additional impact of being born intrauterine growth restricted (IUGR) on the normal aging mechanisms of vascular dysfunction is not known. We hypothesised that IUGR would cause changes in vascular function that would affect the mechanisms of endothelium-dependent vasodilation.

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

Methods: To create an IUGR model, pregnant Sprague Dawley rats were placed in a hypoxic (12% O_2) or control (room air, 21% O_2) environment from day 15 to 21 of the pregnancy. Both male and female offspring were investigated at 4 or 12 months of age. Endothelial function was assessed in small mesenteric arteries using wire myography to study methacholine (MCh)-induced vasodilation. The involvement of nitric oxide (NO), prostaglandins and endothelium-derived hyperpolarising factor (EDHF), was assessed using the inhibitors L-NAME, meclofenamate or a combination of apamin and TRAM-34 (SK_{Ca} and IK_{Ca} blockers) respectively. EDHF-induced vasodilation was further investigated using inhibitors of P450 epoxygenases (MS-PPOH) and gap junctions (18α-GA). All protocols were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee in accordance with the Canadian Council on Animal Care guidelines.
Results: Interestingly, NO mediated vasodilation was not only decreased in aged male and female, control and IUGR rats but was also decreased in IUGR females at a young, 4 month time point. EDHF-mediated vasodilation was maintained in all groups, however, an additional involvement of gap junctions was found in IUGR females which may represent a compensatory mechanism. In contrast in males, gap junctions were involved in vasodilation in both young control and IUGR and aged control animals. Therefore, age (reduction in NO-mediated mechanisms) or the combination of age and hypoxia *in utero* (reduction in both NO- and MEGJ-mediated mechanisms) compromised the diversity of vasodilator pathways in male offspring.
Conclusions: In summary, compromised growth *in utero* was found to affect the mechanisms of vasodilation, reducing the NO component and maintaining an EDHF component. Age alone also decreased the NO component without affecting EDHF-mediated vasodilation. In this study, the loss of NO-mediated vasodilation occurred at an earlier time point in females, but not males, exposed to hypoxia *in utero*. Therefore, while overall vasodilation was maintained, a loss of particular vasodilator pathways such as NO may cause the vasculature to be more susceptible to further dysfunction due to a lack of redundant mechanisms. Interestingly, females, but not males appeared capable of compensating for a decrease in NO function via recruitment of alternative pathways such as gap junctions furthering the hypothesis that females are cardioprotected. JSM was supported by funding from the Strategic Training Program in Maternal, Fetal and Newborn Health (MFN), the Alberta Heritage Foundation for Medical Research (AHFMR) and the Heart and Stroke Foundation of Canada (H&S).

O-9A-82

Vascular dysfunction in children conceived by assisted reproductive technologies

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Objective: Assisted reproductive technologies (ART) has allowed millions of infertile couples to have children who now make up for 1 to 4% of the population in developed countries. ART involves the manipulation of early embryos at a time when they may be particularly vulnerable to external disturbances. Environmental influences acting early in life may predispose to chronic disease in adulthood. The safety of ART for long-term health is, therefore, of utmost importance, but there is little information. Among the potential long-term consequences of ART cardiovascular disease may represent an important problem. The ambient hypoxia associated with high-altitude exposure is known to induce exaggerated pulmonary hypertension and systemic vascular dysfunction in persons displaying endothelial dysfunction. As we anticipated relatively modest vascular dysfunction in young children born after ART, we sought to capitalize on this observation and hypothesized that high-altitude exposure may facilitate the detection of this problem.
Methods: We, studied pulmonary (systolic pulmonary-artery pressure) and systemic vascular function (flow-mediated vasodilation of the brachial artery and pulse wave velocity) in 65 children born from ART (mean age 11 ± 2 y) and 57 age and sex-matched controls during high-altitude exposure (3450 m for 72 hours). Since we observed marked vascular dysfunction at high altitude, the participants were then also studied at low altitude to see whether the dysfunction was also detectable under normoxic conditions at low altitude (542 m).
Results: The major new finding was that at high altitude, the systolic pulmonary-artery pressure was 30 percent higher (39 ± 11 vs. 30 ± 9 mm Hg, $P < 0.001$) and the flow-mediated dilation of the brachial artery roughly 25 percent smaller (6.6 ± 1.8 vs. 8.8 ± 1.7%, $P < 0.001$) in children born after ART than in controls. Pulse wave velocity was significantly faster in children born after ART than in controls, both at the femoral (8.3 ± 1.9 vs. 7.0 ± 1.1 m/sec, $P < 0.0001$) and at the radial (10.2 ± 2.0 vs. 8.3 ± 1.7 m/sec, $P < 0.001$) measurement site. At low altitude, pulmonary-artery pressure, as expected, was not different between the two groups, whereas vascular dysfunction in the systemic circulation was of comparable magnitude as at high altitude. Endothelium-independent vasodilation was similar in the 2 groups (13.5 ± 2.3 vs. 13.9 ± 2.5%, $P = 0.38$, ART vs. controls). Parent-related factors do not appear to contribute importantly to vascular dysfunction in ART children, since vascular function was normal in children who were conceived naturally or with intrauterine insemination following hormonal stimulation of the ovulation in the mother. Maternal age was not associated with more severe vascular dysfunction in the offspring. Oxidative stress was significantly higher in children born after ART than in controls, and there existed a significant relationship between 8-isoprostaglandin F2-α plasma concentration and both, pulmonary-artery pressure ($r = 0.31$, $P = 0.008$) and flow-mediated vasodilation ($r = -0.27$, $P = 0.02$).

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

Conclusions: Offspring of ART display marked vascular dysfunction in both the systemic and the pulmonary circulation early in life. Vascular dysfunction is related to ART itself rather than to parental factors. Exaggerated oxidative stress appears to represent a mechanism underpinning vascular dysfunction in ART. We speculate that offspring of ART are predisposed to premature cardiovascular morbidity and mortality.

O-9A-83

Late gestation uteroplacental insufficiency programs regional vascular dysfunction and alters passive mechanical wall properties in non-pregnant and pregnant growth restricted female offspring

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Objective: Intrauterine growth restriction caused by uteroplacental insufficiency increases the risk of cardiovascular disease in adulthood. Male rat offspring have elevated blood pressure, endothelial and smooth muscle dysfunction and increased arterial stiffness. The aim of this study was to investigate the effects of late gestation uteroplacental insufficiency on regional vascular reactivity and mechanical wall properties in non-pregnant and pregnant adult female rats that had been born growth restricted.
Methods: Uteroplacental insufficiency was induced by bilateral uterine vessel ligation (Restricted) or sham surgery (Control) on day 18 of pregnancy in WKY rats. Restricted and Control non-pregnant female offspring were studied at 4 months of age. Another cohort was mated at 4 months of age and studied on day 20 of pregnancy. Small uterine, renal, mesenteric and femoral arteries were mounted on a wire myograph to assess smooth muscle and endothelial function and on a pressure myograph to assess arterial passive mechanical wall properties. Vascular extracellular matrix gene expression of collagen type I and III ($\alpha 1$) and elastin were determined by quantitative real-time (qRT) PCR. Quantitative collagen and elastin fiber determination, using bright field and cross-polarized light microscopy, are currently in progress.
Results: Blood pressures were not elevated in non-pregnant or pregnant Restricted females. Uterine artery wall stiffness was increased and femoral artery wall stiffness was decreased in Restricted non-pregnant females compared to Controls ($p < 0.05$). Vasoconstriction evoked by angiotensin II and phenylephrine (PE) and total endothelium-dependent or -independent relaxation, or relaxation attributed to endothelium-derived hyperpolarizing factor (EDHF) were not different in arteries from Restricted and Control non-pregnant females. In pregnant Restricted females, there were regional differences in contraction to PE, with upregulation of maximal contraction in mesenteric arteries, while sensitivity was reduced in renal and femoral arteries compared with arteries from pregnant Controls ($p < 0.05$). EDHF-mediated relaxation was impaired in mesenteric and renal arteries from pregnant Restricted females ($p < 0.01$). During pregnancy, uterine artery wall stiffness was normalised in Restricted females, while the mesenteric and femoral arteries were stiffer ($p < 0.05$). Uterine and mesenteric mRNA expression of collagen type I and III ($\alpha 1$) and elastin were not different between groups in non-pregnant or pregnant females.
Conclusions: Uteroplacental insufficiency programs region-specific vascular dysfunction in growth restricted females. The uterine artery is stiffer in non-pregnant Restricted females and this is normalised during pregnancy. The enhanced adaptation of the uterine artery serves to maintain the health of the fetuses, and this is possibly at the expense of other maternal vascular beds in which mechanical and functional deficits emerged.

O-9C-84

Maternal smoking during pregnancy and offspring growth in childhood: 1993 and 2004 Pelotas cohort studies

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Objective: The association between maternal prenatal smoking and offspring low birthweight is well known. However, the consequences of maternal prenatal smoking on offspring weight, length/height and head circumference during childhood are less documented. The objective of this study is to explore offspring growth effects during childhood of maternal smoking during pregnancy using three approaches: 1) multiple adjustments for socioeconomic and parental factors, 2) maternal-paternal comparisons as a test of putative intrauterine effects and 3) comparisons between two birth cohort studies, the 1993 and 2004 Pelotas cohorts, in Brazil.
Methods: Population-based birth cohort studies were carried out in the city of Pelotas, Brazil, in 1993 and 2004 (5304 and 4287 births, respectively). In the 1993 cohort, a systematic sample was examined at home at three (655 infants), twelve and 48 months (1460 and 1450 children, respectively). In the 2004 cohort study, all cohort children were followed-up when they were three, twelve, 24 and 48 months old (3985, 3907, 3869 and 3799 children, respectively). Maternal and paternal smoking behaviour during pregnancy was assessed retrospectively at birth. Multiple linear regression analysis was used to examine the relationships between maternal and paternal smoking and offspring weight, length/height and cephalic perimeter using the following models: unadjusted and adjusted for socioeconomic confounders.

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Maternal and paternal smoking during pregnancy were associated with offspring weight, length/height and head circumference at each stage of follow-up and in both cohorts in the crude analyses. However, maternal smoking associations were much stronger than paternal smoking associations. In the adjusted analyses maternal smoking during pregnancy was associated with weight and head circumference at birth and at three months, but not at later follow-ups, while the association with offspring length/height persisted at each stage of follow-up in both cohort studies (see Figures 1 and 2). Paternal smoking was not associated with any of the growth parameters in the adjusted analyses. Associations were consistent between the Pelotas 1993 and 2004 cohort studies.

Conclusions: Although residual confounding cannot be ruled out, the specificity of the maternal smoking associations with child length/height, the persisting associations following adjustment for multiple confounders, the stronger maternal versus paternal smoking associations, and the consistency between 1993 and 2004 Pelotas cohorts results, all suggest a causal role of maternal smoking in pregnancy on offspring length/height during childhood. Because maternal smoking during pregnancy could compromise offspring growth, which in turn is associated with adult health and human capital, its prevention will bring important health and economic benefits in low and middle income countries.

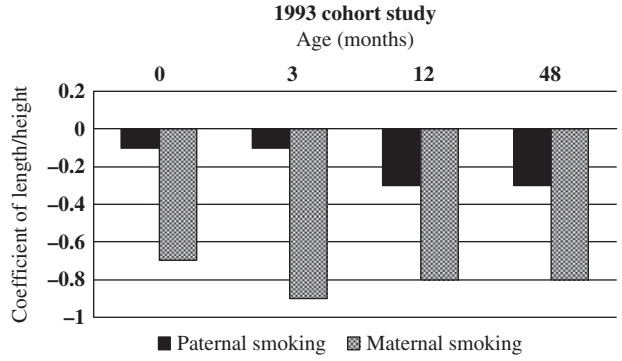

Figure 1: Parental smoking and offspring height in 1993 cohort.

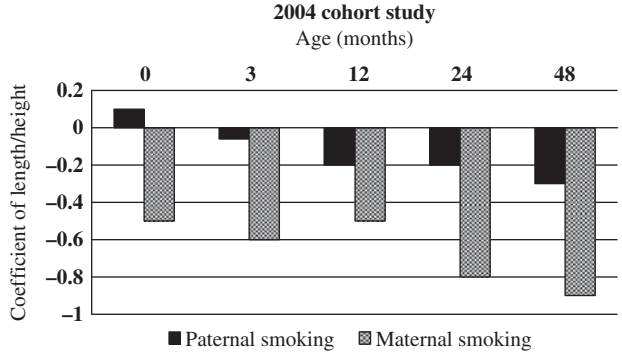

Figure 2: Parental smoking and offspring height in 2004 cohort.

O-9C-85

Periconceptional parental occupational exposure to endocrine disruptors and the risk of congenital heart defects in offspring. The HAVEN study

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Objective: Congenital heart defects (CHDs) are the most common major malformations in newborns and account for over one-third of infant deaths due to congenital anomalies. About 15% of CHDs is attributed to a known cause. The majority of cases are thought to result from complex interactions between genetic susceptibilities and intrauterine exposures mainly derived from the mother. Studies on relationships between occupational exposure and CHD are scarce. The aim of this study was to determine whether periconceptional occupational exposures of the parents-to-be are associated with CHD in the offspring.

Methods: The Dutch HAVEN study of heart anomalies is a case–control study designed to investigate the role of genetic and environmental determinants in the pathogenesis and prevention of CHD. The CHD phenotypes comprised perimembranous ventricle septum defect (pVSD), Tetralogy of Fallot (TOF), atrialventricular septum defect (AVSD), coarctatio aortae (CoA), hypoplastic left heart syndrome (HLHS). From 424 mothers and 421 fathers of a child with CHD and 480 mothers and 477 fathers of a nonmalformed child general characteristics and periconceptional occupational exposure to endocrine disrupting compounds was collected by questionnaires at the child's age of 17 months. Based on job title and description of the job, jobs were coded according to the World Health Organisation standard classification of occupations. A job-exposure matrix was used, linking job title to an expert judgment on exposure to endocrine chemicals at the workplace. In logistic regression analyses univariate associations between maternal and paternal occupational exposure and CHD were studied, expressed by odds ratios (OR) and 95% confidence intervals (95% CI).

Results: Maternal occupational exposure to pesticides and phthalates was significantly associated with CoA, and exposure to polychlorinated compounds, phthalates, alkylphenolic compounds and other chemicals was significantly associated with AVSD. Moreover, periconceptional paternal occupational exposure to polychlorinated compounds was significantly associated with HLHS, exposure to phthalates was associated with pVSD, TOF and HLHS, exposure to alkylphenolic compounds was significantly associated with pVSD, TOF and CoA, and exposure to heavy metals with TOF.

Conclusions: Periconceptional occupational exposure to endocrine disruptors may be associated with different CHD phenotypes. Further research is needed to corroborate these

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

findings, especially in the presence of other potential determinants and to elucidate the actual exposure patterns in jobs.

PERICONCEPTION EXPOSURE	pVSD	TOF	AVSD	CoA	HLHS
MOTHER					
- Pesticides	-	-	2.5 (0.5–11.9)	3.8 (1.0–14.7)*	-
- Polychlorinated cp	-	9.4 (0.6–152.0)	22.8 (2.0–256.8)*	-	-
- Phthalates	1.1 (0.2–5.1)	2.4 (0.5–11.4)	9.3 (3.1–28.2)*	4.3 (1.1–16.9)*	3.1 (0.4–26.1)
- Alkylphenolic cp	0.3 (0.1–1.5)	0.4 (0.05–2.8)	3.6 (1.5–8.9)*	0.9 (0.2–4.0)	1.0 (0.1–7.8)
- Solvents	-	-	3.7 (0.4–36.3)	-	-
- Heavy metals	-	-	2.8 (0.3–25.3)	-	-
- Other chemicals	0.5 (0.1–4.3)	-	4.3 (1.1–16.9)*	2.8 (0.6–13.7)	3.1 (0.4–26.1)
FATHER					
- Pesticides	1.9 (0.8–4.4)	2.0 (0.7–6.1)	1.2 (0.3–5.1)	1.8 (0.5–6.2)	-
- Polychlorinated cp	1.4 (0.7–2.8)	1.7 (0.7–4.1)	1.6 (0.6–4.4)	0.9 (0.3–3.1)	3.4 (1.1–10.7)*
- Phthalates	2.0 (1.1–3.5)*	2.3 (1.1–4.9)*	1.8 (0.8–4.3)	1.8 (0.8–4.3)	4.4 (1.6–12.2)*
- Alkylphenolic cp	2.1 (1.0–4.3)*	4.1 (1.9–9.2)*	1.7 (0.6–5.2)	2.7 (1.1–7.1)*	-
- Solvents	4.3 (0.3–69.0)	9.3 (0.6–151.0)	-	-	-
- Heavy metals	1.6 (0.7–3.9)	3.8 (1.5–9.4)*	0.6 (0.1–4.3)	2.4 (0.8–7.4)	-
- Other chemicals	2.5 (0.7–8.6)	2.7 (0.5–13.3)	1.6 (0.2–13.0)	3.2 (0.6–15.9)	-

*indicates significant p-value. $P < 0.05$. cp, compounds.

O-9C-86

Exposure of pregnant rats to diverse chemicals during pregnancy causes elevated blood pressure in offspring

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Objective: Global undernutrition, low protein diet or dexamethasone treatment during pregnancy has been demonstrated in animal models to result in adverse health effects including hypertension and insulin resistance in adult offspring. Most protocols that produce these effects cause *in utero* growth retardation (IUGR). IUGR is also common in prenatal developmental toxicity studies. Here we examine long term effects of *in utero* chemical exposure.

Methods: Pregnant rats were exposed to chemicals at dosages chosen to result in small decrements in birth weight. Chemicals studied included dexamethasone (positive control), perfluorooctane sulfonate (PFOS), atrazine, arsenic, perfluorononanoic acid (PFNA), nicotine, and toluene. Following parturition, offspring were cross-fostered to untreated dams and their survival and growth monitored. Offspring blood pressure (tail-cuff) and insulin response (ELISA) to oral glucose challenge were measured at several ages. Some offspring were killed on postnatal day 1 for measurement of kidney glucocorticoid receptor mRNA by qRT-PCR and some were killed at day 22 to collect kidneys for nephron counts by confocal microscopy. At the end of the study, animals were killed and serum chemistries assessed.

Results: Chemical exposure during pregnancy resulted in birth weights significantly lower than controls for dexamethasone, atrazine, and PFNA, and nominally lower for PFOS and nicotine. Offspring weights were similar to control by weaning except for dexamethasone. At 7-10 weeks of age, systolic blood pressure was higher than controls in male offspring of dams exposed to dexamethasone, atrazine, PFOS, PFNA, and toluene and significantly higher in females for PFNA and arsenic. Females in general were affected later than males; PFOS, dexamethasone and atrazine female had elevated blood pressures by 37 weeks. By 52–65 weeks of age, both males and females showed elevated blood pressure for dexamethasone, PFOS, and atrazine, as did arsenic males. Nicotine, PFNA and toluene groups had blood pressures similar to controls at 56 weeks. Nephron counts revealed significant deficits in the dexamethasone, PFOS, PFNA, and atrazine groups. Kidney glucocorticoid receptor mRNA was elevated in the dexamethasone, PFOS, and atrazine groups. There were no consistent effects on insulin response to oral glucose, nor was there any indication of obesity as indicated by body weights. There were sporadic effects on serum chemistry.

Conclusions: Diverse chemical exposures to pregnant rats caused elevated blood pressure in offspring. Lower nephron endowments and elevated glucocorticoid receptor expression may contribute. IUGR and/or maternal stress may be common to all of these exposures.

O-9C-87

Smoking and alcohol use affects first trimester fetal growth. The Generation R Study

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Objective: Adverse exposures during the periconceptional and fetal period may be associated with early developmental adaptations and with an increased risk of cardiovascular and metabolic disease. Not much is known about adverse exposures affecting early fetal development. First trimester crown-rump length (CRL) may be used for assessing embryonic and early fetal development among mothers with a well-known gestational age. Therefore, we examined whether periconceptional smoking and alcohol use are associated with first trimester fetal growth variation.

Methods: This study was embedded in a population-based prospective cohort study. CRL was measured in the first

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

trimester of pregnancy by fetal ultrasound. Gestational age was determined by means of last menstrual period. Only mothers with a regular menstrual cycle of 24–32 days were included in the present analysis. Periconceptional smoking and alcohol use were assessed through questionnaire. Analyses were performed on 1,936 mothers and fetuses. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Median gestational age based on last menstrual period was 12.6 weeks (90% range: 10.9–14.9). Periconceptional smoking and alcohol use were associated with a decrease in CRL of 1.55 mm (95% CI: 0.52, 2.59) and 1.33 mm (95% CI: 0.10, 2.56), respectively. The effect of smoking remained after adjusting for maternal age, educational level, ethnicity and parity, though the effect of alcohol use disappeared.

Conclusions: Maternal smoking affects early fetal growth and development. Longer follow-up studies are necessary to determine whether these changes in early fetal growth persist throughout pregnancy and are related to adverse outcomes in later life.

O-10A-88

Oral vitamin B_{12} supplementation at physiological doses lowers plasma homocysteine concentration: A cluster-randomized, placebo-controlled, double-blind trial in a rural Indian community

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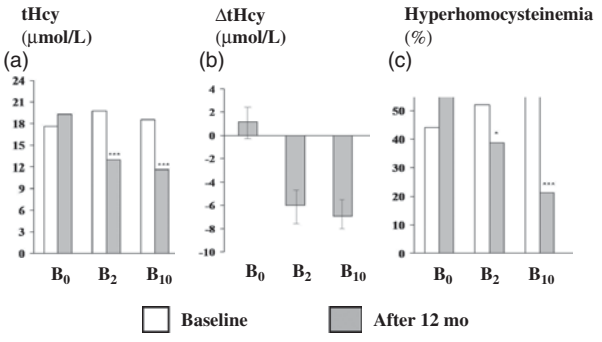
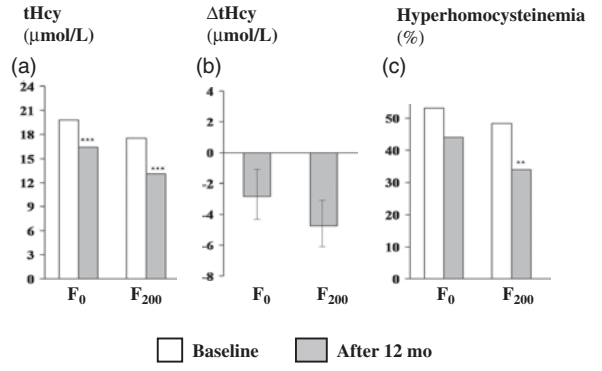
Objective: Vitamin B_{12} (B_{12}) deficiency is common in Indians and a major contributor to hyperhomocysteinemia, which may influence fetal growth, cognitive function, risk of type 2 diabetes and cardiovascular disease. Our objective was to study the effect of physiological doses of B_{12} and folic acid on plasma total homocysteine (tHcy).

Methods: A cluster randomized, placebo-controlled, double-blind, 2×3 factorial trial, using the family as the randomization unit. Vitamin B_{12} was given as 2 or 10 μg capsules, with or without 200 μg folic acid, forming six groups (B_0F_0, B_2F_0, $B_{10}F_0$, B_0F_{200}, B_2F_{200}, $B_{10}F_{200}$). Plasma tHcy concentrations were measured before and after 4 and 12 mo of supplementation. KEM Hospital Research Centre's ethics committee clearance, parents' informed, written consent and children's assent were obtained.

Results: Three hundred individuals from 119 families were randomised. There was no interaction between B_{12} and folic acid ($P = 0.14$) in relation to tHcy change, and their effects were analyzed separately: B_0 vs. B_2 vs. B_{10} and F_0 vs. F_{200}. The baseline-adjusted fall in tHcy was 5.9 (95% CI: −7.8, −4.1) μmol/L in the B_2 group and 7.1 (95% CI: −8.9, −5.4) μmol/L in the B_{10} group (not significantly different). The B_0 group showed a non-significant rise of 1.2 (95% CI: −0.5, 2.8) μmol/L. After 12 mo, in the B_2 group the proportion of hyperhomocysteinemic participants fell from 52% to 39% ($P = 0.02$), and in the B_{10} group fell from 56% to 21% ($P < 0.000$). In the B_0 group it increased from 44% to 56% ($P = 0.02$) (Fig 1). The fall in tHcy concentration in F_0 and F_{200} group was similar: in F_0 group it was 2.8 (95% CI: −4.3, −1.2) μmol/L and in the F_{200} group it was 4.8 (95% CI: −6.3, −3.3) μmol/L. In the F_0 group the proportion of hyperhomocysteinemic participants fell from 53% to 44%, $P = 0.07$ and in the F_{200} group from 48% to 34%, $P = 0.003$ (Fig 2).

Conclusion: Daily oral supplementation with near physiological doses of B_{12} is an effective community intervention to reduce plasma tHcy in a rural Indian population. Folic acid (200 μg/d) showed no additional benefit, neither had any unfavourable effects**.** Our findings provide the evidence to plan a public health intervention to improve maternal B_{12} status in India. Trial Registration: ISRCTN 59289820. Funding source: The Wellcome Trust, UK.





https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

O-10A-89

Maternal folate and homocysteine levels in early pregnancy affect third trimester fetal arterial resistance. The Generation R Study

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Objective: Low birth weight is associated with cardiovascular disease in adulthood. Low maternal folate and high maternal homocysteine might lead to both fetal growth restriction and an adverse cardiovascular development. The aim of this study was to examine the associations of maternal folate and homocysteine levels in early pregnancy with third trimester adaptations in fetal arterial resistance measures and birth weight.

Methods: This study was embedded in a population-based prospective cohort study from early fetal life onwards. First trimester maternal folate and homocysteine levels were categorized in risk groups: the lowest 5th percentile of folate levels and the highest 95th percentile of homocysteine levels. Placental and fetal cerebral and cardiac blood flow and fetal growth characteristics were assessed by ultrasound and Doppler in third trimester of pregnancy. Analyses were based on 892 children. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Offspring of mothers with low (<5th percentile) folate levels and high (>95th percentile) homocysteine levels had increased umbilical artery resistance (0.06 (95% CI: 0.01, 0.11) and 0.04 (95% CI: 0.01, 0.07), respectively). Furthermore, we found an association between these risk groups and an increased mitral valve E/A-ratio (0.05 (95% CI: 0.02, 0.08) and 0.02 (95% CI: 0.01, 0.03), respectively), suggesting adaptations in cardiac diastolic filling patterns. No associations were found with other fetal arterial resistance indices. We observed trends for positive and inverse associations of continuously measured maternal folate and homocysteine levels with birth weight ($p < 0.05$). Offspring birth weight was lower among mothers with low folate and high homocysteine levels (−165.0 grams (95% CI: −313.2, −16.7) and −140.1 grams (95% CI: −269.1, −11.1), respectively, compared to the reference groups). All analyses were adjusted for gestational age, gender and estimated fetal weight.

Conclusions: Low plasma folate levels and high homocysteine levels were associated with both cardiac and arterial resistance adaptations and fetal growth retardation. Our results suggest that folate and homocysteine levels in early pregnancy may be involved in the associations between low birth weight and cardiovascular disease. Further studies are needed to identify the underlying epigenetic and developmental mechanisms and to examine whether these relations persist during later life and are associated with the development of cardiovascular disease.

O-10A-90

Folate depletion during development: consequences for DNA methylation and gene regulation

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Objectives: Growing evidence from animal models suggests a variety of nutritional insults *in utero* result in altered programming of offspring, ultimately causing increased disease risk in later life. In a previous study, we observed that maternal folate depletion during pregnancy pre-disposed mice to be heavier in adulthood ($p = 0.016$, unpublished data). Folate depletion may influence DNA methylation at CpG dinucleotide sites through effects on the supply of the methyl donor *S*-adenosylmethionine, providing one candidate mechanism to explain developmental programming in this model. Indeed, we observed previously that genomic DNA methylation was lower in adult offspring born to folate depleted dams ($p = 0.010$, unpublished data). We aim to investigate the influence of maternal folate depletion on developmental programming, focusing upon DNA methylation as a candidate mechanism.

Methods: Pairs of female C57BL/J6 mice were assigned randomly to a folate-adequate (2 mg folic acid/kg body weight) or folate-deplete (0.4 mg folic acid/kg body weight) diet 5 weeks prior to timed mating with a C57BL/J6 male. Dams remained on allocated diets until day 17.5 gestation, when dams were killed and fetuses removed and weighed. Fetal livers were removed, weighed and snap frozen. DNA and RNA were extracted simultaneously from fetal livers. To identify genes regulated by maternal folate depletion, hepatic RNA from male fetuses was hybridised to NuGO Affymetrix mouse whole genome arrays. A bioinformatic approach[1] was developed to identify genes for which changes in DNA methylation may have mediated responsiveness to folate supply, some of which were analysed for promoter DNA methylation using pyrosequencing technology. Currently we are optimising a microarray-based technique to investigate DNA methylation. Methylated DNA is immunoprecipitated then amplified by PCR, before hybridisation to Roche NimbleGen Methylation 385 K arrays to identify regions of the genome that are methylated differentially between test and control groups.

Results: In male fetal livers, 680 genes were differentially expressed (321 up-regulated, 359 down-regulated; fold change of ±1.2 and $p < 0.05$). Using pathway analysis, we identified genes involved in fatty acid and lipid metabolism,

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

which were differentially expressed in the liver of folate-deplete offspring. Six up-regulated genes – *Acyl, Ehhadh, Irs2, Ppap2a, Rxra, Scd1* – were present in the majority of these pathways. All 6 genes have CpG islands, including transcription factor binding sites that incorporate CpG dinucleotides, present within their promoters, consistent with regulation by DNA methylation. Methylation of the *Scd1* promoter was lower ($p < 0.05$) in livers of folate-deplete offspring.

Conclusions: Folate depletion during pregnancy can alter gene expression in the mouse fetal liver, concomitant with pre-disposing offspring to be heavier in adulthood and increasing expression of genes involved in fatty-acid and lipid metabolism. Effects on DNA methylation may be one mechanism underlying such effects. Supported by NuGO and the BBSRC (BB/G007993/1).

1. J.A. McKay *et al.*, *Genes Nutr.,* 3:167-171, 2008.

O-10A-91

Maternal early pregnancy vitamin D status in relation to fetal and neonatal growth: results of the ABCD study

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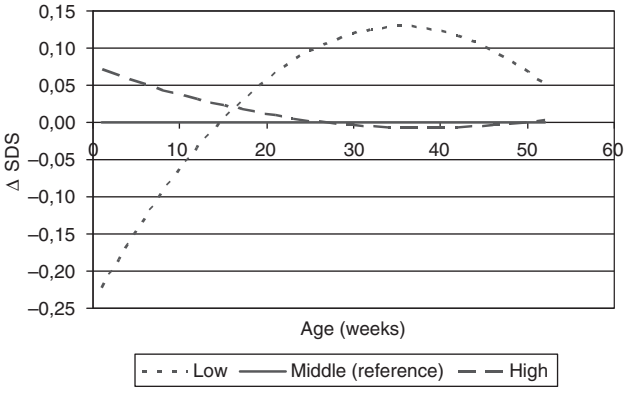
Objective: Adequate maternal nutrition has since long been accepted as vital to adequate fetal, and consequently, neonatal development. However, it is still largely unclear to what extent which nutrients matter. An important vitamin that is recognized as a possible influential component is vitamin D. The present study explored the association of maternal vitamin D levels in early pregnancy with birth weight and neonatal weight in the first year of life.



Methods: Data from the Amsterdam Born Children and their Development (ABCD) cohort study were used. Pregnant women donated blood during their first antenatal visit (median 13 weeks, IQR 12–14) and subsequently filled out an extensive questionnaire. Serum vitamin D levels were measured by an enzyme-immunoassay method. Infant birth weight, gestational age, and gender, as well as neonatal weight at the age of 1, 3, 6, 9 and 12 months were collected from youth health care centres. Neonatal weight was transformed into standard deviation scores (SDS). Associations of vitamin D levels (low: ⩽29.9 nmol/L; intermediate: 30–49.9 nmol/L; high: ⩾50 nmol/L) with birth weight and neonatal weight were examined by univariate and multivariate linear regression analyses (all standardized for season of vitamin D measurement). Only mothers with live born singleton term deliveries were included (n = 3730). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Women with low vitamin D levels (23%) delivered infants with a lower birth weight (estimated difference −87.2 g, 95% CI: −108.1;−20.8), while women with high vitamin D levels (56%) tended to have infants with a higher birth weight (35.5 g, 95% CI: −1.9;73.0), as compared to women with intermediate levels (21%). After adjustment for maternal physiologic, sociodemographic, and behavioural factors, low vitamin D levels remained significantly associated with birth weight (−64.4 g, 95% CI: −108.1; −20.8). Low vitamin D, but not high vitamin D, was also associated with neonatal growth, with lower weight SDS until month 3, and higher SDS thereafter (figure).

Conclusions: Low maternal vitamin D status in early pregnancy was associated with lower birth weight and altered neonatal growth. The observed catch-up growth suggests that infants born to mothers with a low vitamin D status are at higher risk for developing metabolic and cardiovascular disease in later life. Although in the Netherlands pregnant women are recommended to increase their intake of vitamin D, this advice is still not fully implemented. Our study indicates that supplementation might be beneficial for those at risk of low vitamin D levels, but more research is needed before a nationwide policy can be justified. Financial support was granted by the Netherlands Organisation for Health Research and Development and the National Institute for Public Health and the Environment.

O-10B-92

Cross-sectional and longitudinal associations between physical activity and blood pressure in adolescence: birth cohort study

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Objective: To explore cross-sectional and longitudinal associations between physical activity (PA) and blood pressure (BP) between 11 and 14 years of age, and to evaluate whether accelerometer-measured and questionnaire-based PA are differently associated with BP in adolescence.
Methods: Prospective birth cohort study in Pelotas, a 340,000-inhabitant city in Southern Brazil. The study included 427 members of the birth cohort who were followed up at 11, 12 and 14 years of age, and had questionnaire data on PA and BP at 11 and 14 years, as well as accelerometry and questionnaire data on PA at 12 years. Outcome variables were continuous systolic and diastolic BP at 14 years, and change in BP from 11 to 14 years. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: PA was not related to systolic BP in any analyses. PA measured by accelerometry at 12 years, but not questionnaire-derived PA, was inversely associated with diastolic BP at 14 years of age in fully adjusted models. Adolescents who were categorized as being physically active (>300 min/wk) by questionnaire for all visits (11, 12 and 14 years) had a 2.6 mmHg lower mean increase in diastolic BP from 11 to 14 years compared to those not achieving 300 min/wk.
Conclusions: Accelerometry-based PA was longitudinally inversely associated with diastolic, but not systolic BP indicating a beneficial effect of PA in early adolescence on BP development. This finding was not evident when analysing self-reported PA, indicating that assessing PA using questionnaires may underestimate its beneficial effects on metabolic phenotype. This analysis was supported by the Wellcome Trusts initiative entitled Major Awards for Latin America on Health Consequences of Population Change. Earlier phases of the 1993 cohort study were funded by the European Union, the National Program for Centers of Excellence (Brazil), the National Research Council (Brazil) and the Ministry of Health (Brazil).

O-10B-93

Late, but not early, exercise restores markers of mitochondrial biogenesis in skeletal muscle of growth restricted rats

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Objective: Fetal growth restriction induced by uteroplacental insufficiency in rats results in impaired glucose tolerance and is associated with reduced markers of skeletal muscle mitochondrial biogenesis in adulthood, particularly in males. Exercise training is well known to increase mitochondrial biogenesis in skeletal muscle. We therefore determined whether brief juvenile or adult exercise training restored protein expression of mitochondrial biogenesis markers in skeletal muscle in growth restricted male rats.
Methods: Uteroplacental insufficiency was induced on day 18 of pregnancy and resulted in growth restricted (*Restricted*) litters which were compared to sham-operated *Controls*. Furthermore, to control for the reduction in litter size in *Restricted* litters, sham-operated *Reduced litter* offspring were included, where litter size was reduced at birth, previously reported to impair postnatal lactation and growth. Offspring remained sedentary or underwent Early (5–9 weeks) or Late (20–24 weeks) exercise training. Exercise training involved treadmill running 5 days/week, 60 minutes/day. We examined offspring growth and skeletal muscle protein expression of mitochondrial biogenesis markers (PGC1α, Tfam, CytC, COX IV and NRF2) at either 9 or 24 weeks by western blot analysis ($n = 8$/group).
Results: *Restricted* offspring were smaller than sham operated offspring 1 day after birth and remained smaller until 16 weeks of age when the *Control* and *Restricted* offspring were not different from each other but were significantly smaller than *Reduced litter* offspring ($P < 0.05$). There was no effect of early exercise on body weight at any age, however, at 24 weeks of age rats that underwent late exercise were lighter than those who remained sedentary or underwent early exercise training ($P < 0.05$). Early exercise training increased PGC1α protein expression at 9 weeks of age ($P < 0.05$) but was not different between *Control*, *Restricted* and *Reduced litter* groups. *Restricted* and *Reduced litter* offspring had lower PGC1α protein expression at 24 weeks of age compared with *Control* offspring ($P < 0.05$). Late, but not early, exercise increased PGC1α protein expression at 24 weeks of age ($P < 0.05$). Protein expression of Tfam and Cytochrome C were not different between any groups at 9 weeks of age. Late, but not early, exercise increased Tfam and Cyt C protein expression at 24 weeks of age ($P < 0.05$) but were not different between groups. There were no differences in NRF2 and COX IV protein expression in any groups at both ages regardless of exercise.
Conclusion: In conclusion, early life exercise training increases skeletal muscle PGC1α protein expression regardless of growth restriction but the effect was not sustained into adulthood after cessation of exercise. Late exercise training also increased some, but not all, markers of mitochondrial biogenesis in skeletal muscle. This finding is important for growth restricted offspring who have lower PGC1α expression at 24 weeks of age and may contribute to preventing the onset of type 2 diabetes.

O-10B-94

Swim training applied at early age is critical to attenuate monosodium L-glutamate-programmed obesity onset in mice

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https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

Objective: Exercise has been recommended as a remedy against a worldwide obesity epidemic; however, the onsets of excessive weight gain is not fully understood, nor are the effects of exercise on body weight control. Changes on brain areas which control body weight related to activity deficits of the sympathetic nervous system, including the sympathoadrenal axis, have been suggested to contribute to high fat accumulation in obesity; however, it has been suggested that early exercise may program metabolism to resist to overweight onset. In the present work, swim training was used to observe fat accumulation and adrenal catecholamine stocks in hypothalamic-obese mice produced by neonatal treatment with monosodium L-glutamate (MSG).
Methods: MSG-treated and normal mice swam for 15 min/day, 3 days a week, from weaning up to 90 days old (EXE 21-90); from weaning up to 50 days old (EXE 21-50) and from 60 up to 90 days old (EXE 60-90). Sedentary MSG and normal mice (SED groups) did not exercise at all. Animals were sacrificed at 90 days of age.
Results: MSG treatment induced obesity, demonstrated by a 43.08% increase in epididymal fat pad weight; these adult obese mice presented 27.7% less catecholamine stocks in their adrenal glands than untreated mice ($p < 0.001$). Exercise reduced fat accumulation and increased adrenal catecholamine content in EXE 21–90 groups. These effects were more pronounced in MSG-mice than in normal ones. Halting the exercise (EXE 21–50 groups) still changed fat accretion and catecholamine stocks; however, no effects were recorded in the EXE 60–90 groups.
Conclusions: Metabolic changes imposed by early exercise with low intensity and frequency, leading to an attenuation of MSG-hypothalamic obesity onset, are at least in part due to a changes on sympathoadrenal activity modulation. Financial support: CNPq/CAPES/Fundação Araucária.

O-10B-95

Relationship between physical activity measured using accelerometers and energy expenditure measured using doubly-labelled water in Indian children

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Objective: To test the association between physical activity measured using accelerometer counts (Actigraph), and energy expenditure measured using the doubly-labelled water method in free-living children in India. The aim was to explore the usefulness of Actigraphs for estimating energy expenditure.
Methods: Total energy expenditure (TEE) was measured in 58 children aged 8–9 years over a period of 2 weeks using the doubly-labelled water (DLW) technique. Physical activity level (PAL) was estimated from TEE and basal metabolic rate predicted from weight. Physical activity was measured simultaneously using the Actigraph accelerometers (MTI AM7164 and GT1M). TEE was also calculated from the Actigraph counts using a published equation. The study was approved by the Holdsworth Memorial Hospital, Mysore, research ethics committee and informed consent was obtained from parents and children.

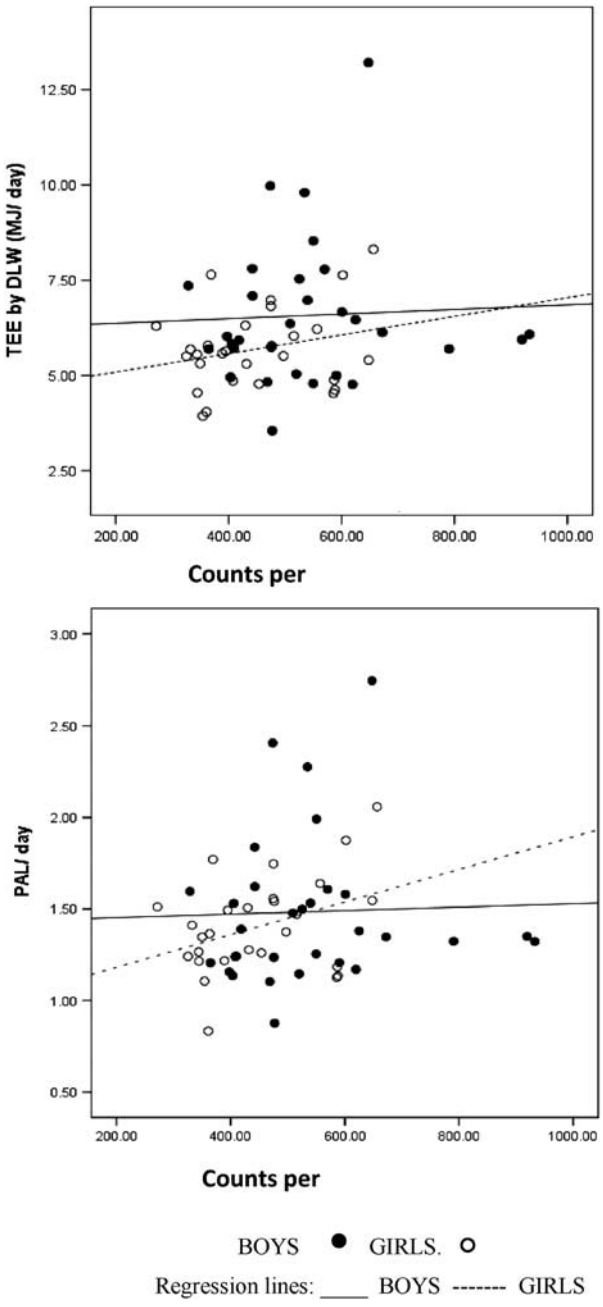

Figure 1. Scatter plot showing association between Actigraph counts and TEE and PAL from the DLW technique (stratified by sex).

Results: TEE (Median: 6.1 MJ v 5.6 MJ, $P = 0.04$) and Actigraph counts (counts per minute: 522.6 v 430.7,

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

P = 0.01; total counts: 414040 v 329756, P = 0.002) were higher in boys compared to girls. There were no significant correlations between either total Actigraph counts (r = 0.15, P = 0.3) or counts per minute (0.18, P = 0.2) and TEE estimated using DLW (Figure 1). Similarly, there were no significant correlations between Actigraph counts and PAL (r = 0.10, p = 0.5; r = 0.17, p = 0.2). Bland-Altman analysis showed poor agreement between TEE estimated using DLW and TEE derived from the Actigraph equation.

Conclusions: Activity measured using Actigraph accelerometers is not related to TEE and PAL derived using the DLW technique in Mysore children. Actigraphs may not be useful in predicting energy expenditure in this setting. Funding source: The study was supported by the Parthenon Trust, Switzerland, the Wellcome Trust and the Medical Research Council, UK.

O-10C-96

Prenatal- and postnatal growth and trait anxiety in late adulthood – the Helsinki Birth Cohort Study

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Objective: Anxiety predisposes to cardiovascular events. These conditions may share a common origin in a suboptimal prenatal and postnatal environment, which could be reflected in alterations in body size at birth and in subsequent growth. We tested whether prenatal- or postnatal growth from birth to 11 years of age, and body size in adulthood predict trait anxiety, a propensity to experience anxiety intensely and frequently, in late adulthood in participants of the Helsinki Birth Cohort 1934–44 Study.

Methods: Women (n = 951) and men (n = 753) born between 1934 and 1944 reported trait anxiety using Spielberger Trait Anxiety Scale at an average age of 63.4 years. Growth in weight, height, and BMI was estimated from birth, child welfare clinic, and school health records monthly from birth to two years and annually from seven to 11 years, and adult body size was measured during a clinical visit. In addition, we identified two factor analytically derived uncorrelated composite measures (components) to characterize the prenatal pattern of growth. Multiple linear regression analyses were used to test the association between all growth measures and trait anxiety in adulthood. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Higher trait anxiety was predicted by composite score reflecting smaller overall body size at birth and gestational age, lower weight and smaller body mass index (BMI; kg/m^2) at birth and in infancy, and shorter height and lower weight in adulthood (all standardized β's ≤ -0.05, 95% Confidence Intervals [CI] -0.11 to 0.00, p-values < 0.04). Moreover, higher trait anxiety was predicted by faster growth in weight particularly from seven to 11 years of age (standardized $\beta = 0.05$, 95% CI 0.00 to 0.10, p = 0.04) as well as a smaller difference in weight and height between 11 and 63 years (all standardized β's ≤ -0.06, 95% CIs -0.13 to -0.01, p-values < 0.02). These results remained after adjusting for major confounders.

Conclusions: We found a pattern of prenatal and postnatal growth that predisposed to higher trait anxiety in late adulthood. This pattern resembles that found to increase the risk for cardiovascular events, and thus, points to a shared common origin in a suboptimal prenatal and childhood developmental milieu.

O-10C-97

Childhood separation experience predicts hormonal response at age 60 to 70: a natural experiment in World War II

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Animal models have linked early life adversity with life-long changes in hypothalamic-pituitary-aderenal-axis (HPAA) activity. Human evidence is mostly based on clinical samples.

Objectives: World War II created a natural experiment to test whether evacuation to temporary foster care unaccompanied by either parent is associated with alterations in HPAA activity to psychosocial stress in late adulthood.

Method: 287 participants of the Helsinki Birth Cohort Study 1934–1944 underwent the Trier Social Stress Test (TSST) during which we measured salivary cortisol and, for 215 individuals, plasma cortisol and ACTH concentrations.

Results: Evacuees had higher overall salivary cortisol and plasma ACTH levels compared to the non-separated, and

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press

higher salivary cortisol reactivity to the TSST. Salivary cortisol reactivity was higher among those evacuated for longer durations and in early childhood.
Conclusions: Early life adversity induces alterations in an individual's stress physiology that may last over the life-course.

O-10C-98

Growth in infancy and childhood and hospitalization for personality disorders in adulthood

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Objective: While previous studies have shown associations between suboptimal prenatal growth and personality disorders in adulthood, studies on the associations between infant and childhood growth and personality disorders are lacking. Hence, the aim of this study was to examine the associations between infant and childhood growth and hospitalisation for personality disorders.
Method: The sample consisted of 4689 men and 4200 women in the Helsinki Birth Cohort Study 1934–1944 who had adequate data including growth data from birth to 11 years from birth, child welfare, and school records and data on the diagnoses of personality disorders from the national Hospital Discharge Register. 81 men and 68 women had been hospitalised with an ICD (−8, −9, −10) diagnosis of personality disorder. Appropriate institutional ethics committee clearance was obtained.
Results: After adjusting for socioeconomic position of family of origin and for year of birth, we found that among men, faster gain in weight (Odds Ratio (OR) for each standard deviation increase in growth = 1.36, 95% Confidence Interval (CI): 1.10–1.68, p = 0.004) and in BMI between 6 months and 1 year (OR = 1.27, 95% CI = 1.04–1.56, p = 0.02) and slower gain in weight (OR = 0.70, 95% CI = 0.55–0.89, p = 0.004) and in BMI (OR = 0.68, 95% CI = 0.54–0.85, p = 0.001) between 7 and 11 years of age predicted increased risk of hospitalisation for personality disorders in adulthood. Thinness at six months (OR = 0.78, 95% CI = 0.62–0.98, p = 0.03) and again at 11 years of age (OR = 0.77, 95% CI = 0.62–0.96, p = 0.02) also showed significant association with personality disorders among men. Among women, slower gain in height between 2 and 7 years of age (OR = 0.76, 95% CI = 0.59–0.97, p = 0.03) predicted hospitalisation for personality disorders.
Conclusions: Our findings point to the importance of early growth as a vulnerability factor of hospitalisation for personality disorders in adult life.

O-10C-99

Depression and anxiety after prenatal exposure to the Dutch famine

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Objective: There is increasing evidence that an adverse prenatal environment may predispose to the development of depression and anxiety in later life. We investigated whether prenatal exposure to the Dutch famine increases the risk for depression and anxiety.
Methods: A total of 819 men and women, born as term singletons around the 1944–1945 Dutch famine, filled out the Hospital Anxiety and Depression Scale (HADS) and participated in a standardized interview on medical history and lifestyle. Results were compared between men and women prenatally unexposed (*n* men = 232, *n* women = 253) and exposed to famine during late (*n* = 61, *n* = 77), mid (*n* = 45, *n* = 76) or early gestation (*n* = 31, *n* = 44). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Men exposed to famine during early gestation had a higher total depressive symptom score than unexposed men (median scores 4 vs. 2, *P* = 0.01). Also, the percentage of men ever clinically diagnosed with depression tended to be higher among those exposed to famine during early gestation than among those unexposed (OR 2.6 [95% CI: 0.8–8.5]). Mild to severe anxiety symptoms (HADS-A score ⩾8) were more prevalent among early exposed men compared to unexposed men (OR 2.7 [1.2–6.1]) and their total anxiety symptom score tended to be higher (5 vs. 4, *P* = 0.08). All results were independent of birth weight and presence of chronic diseases including hypertension, type 2 diabetes and coronary heart disease.
Conclusions: Although numbers were small, our results suggest an increased risk of depression and anxiety for men but not women exposed to famine in early gestation.

https://doi.org/10.1017/S2040174409990018 Published online by Cambridge University Press