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PI-001

The Developmental Priming of Fatty Liver Disease Involves *SIRT1* Reduction, Clock Gene Misalignment, and Lipogenic Transcription Factor Up-Regulation. Kimberley D. Bruce, Kiran K. Sihota, Nikesh R. Patel, Mark A. Hanson, Christopher D. Byrne, Felino R. Cagampang. *Human Development and Health, University of Southampton, United Kingdom.*

In the UK 16% of women are obese during pregnancy. We have previously shown that maternal high fat (HF) exposure primes the adult onset of severe fatty liver disease; through mitochondrial dysfunction and elevated lipogenesis. Since hepatic lipid homeostasis is regulated by the molecular "clock" and downstream lipogenic transcription factors (LTF), we hypothesize that perturbations in this system could contribute to the developmental priming of fatty liver. Therefore, we investigated the effect of pre- and postnatal HF exposure on *BMAL1,* and LTF (*RevErb*,) gene expression in adult offspring. We also determined *SIRT* expression; an NAD+ dependant CLOCK/BMAL regulator, and thus a functional link between metabolism and "clock."

Female mice were fed a HF or control chow (C,) diet before and during pregnancy and lactation. Resulting offspring were fed either a C or HF diet after weaning to generate four offspring groups; HF/HF, HF/C, C/HF, C/C. Livers from 15 weeks old male offspring (n=5 per group) were taken during the day and night. Total RNA was extracted and quantitative PCR was used to determine relative clock and clock-related gene expression.

In C/C offspring *BMAL1* expression was 13.8 fold higher ($p<0.0001$) at night. This pattern was maintained in C/HF and HF/C. However, HF/HF daytime *BMAL1* expression became elevated ($p=0.029$), reversing this pattern. *RevErb* was 6.7-fold higher ($P=0.003$), during the day in C/C, whilst this pattern diminished (C/HF 3.2-fold, HF/C 1.6 fold, HF/HF 1.2 fold) through prolonged high fat exposure. *SIRT1* expression was higher in the day in C/C, however this pattern was lost in C/HF and HF/C and the overall expression was lower ($p=0.023$) in HF/HF offspring.

Our results demonstrate that HF diets during early development and in post weaning life can cause circadian perturbations and metabolic dysfunction. Specifically, combined HF exposure during development *and* adulthood elevates *BMAL1* expression, which contributes to the downstream elevation of *RevErb*, thus directly affecting hepatic lipid homeostasis. We suggest that *BMAL1* elevation may at least in part be due to reduced *SIRT1*, secondary to mitochondrial dysfunction and NAD+ reduction. Identification of HF induced *SIRT1-BMAL-RevErb* misalignment provides mechanistic insights into the developmental priming of fatty liver.

PI-002

Intergenerational Transmission of Growth Restriction, Nephron Deficits and Hypertension in Rats. Linda A. Gallo[1], Karen M. Moritz[2], Melanie Tran[1], Marc Q. Mazzuca[1], Luise A. Cullen-McEwen[3], Kate M. Denton[4], Mary E. Wlodek[1]. *[1]Physiology, The University of Melbourne, Australia; [2]Biomedical Sciences, University of Queensland, Australia; [3]Anatomy and Developmental Biology, Monash University, Australia; [4]Physiology, Monash University, Australia.*

Intrauterine growth restriction increases risk of adult disease, particularly in male offspring, with recent evidence for transmission to subsequent generations. We determined whether pregnancy in growth restricted females unmasks cardiorenal alterations that are otherwise absent and whether growth restriction, nephron deficits, hypertension and renal dysfunction associated with uteroplacental insufficiency are transmitted to the next generation.

Late gestation uteroplacental insufficiency was induced by bilateral uterine vessel ligation (Restricted, R) or sham surgery (Control, C) in WKY rats. At 4 mo, Restricted and Control female offspring (F1) were mated with normal males. In F1 pregnant dams, blood pressure (tail-cuff; E18), 24h renal excretions (E19-20) and glomerular number and volume (E20) were assessed. F2 fetal weight and nephron number (E20) were determined. In a separate cohort, F2 offspring were aged to six or 12 mo for blood pressure (tail-cuff) and 24h renal excretion. In F2 males aged to 12 mo, MAP (indwelling tail artery catheter) and renal function (^{3}H-inulin and ^{14}C-PAH clearance) were also measured.

Maternal blood pressure and renal excretions during pregnancy were not different, despite a 37% reduction in F1R nephron number ($P<0.05$) and concomitant glomerular hypertrophy ($P<0.05$). F2R male and female fetuses were smaller than F2C ($P<0.05$). F2R male fetuses had 20% fewer nephrons ($P<0.05$), with female analysis ongoing. At 6 mo, F2R males had increased systolic blood pressure (150±4 vs. 135±6 mmHg; $P<0.05$) that was not evident at 12 mo. Female blood pressure was not different at six or 12 mo. Renal excretions and function were not different at six or 12 mo.

We provide novel evidence for intergenerational transmission of fetal growth restriction in male and female offspring, as well as nephron deficits confirmed in males. Elevated blood pressure observed at 6 mo in F2 Restricted males only, was absent by 12 mo and adult renal function was normal in both genders. These data suggest that while adult cardiorenal function appears normal by 12 mo in offspring born to growth restricted mothers, the transmission of growth and nephron deficits may increase susceptibility to adverse life style challenges.

PI-003

Implication of the mTOR Pathway in Pancreatic β-Cell Programming. Emilyn U. Alejandro[1], Lynda Elghazi-Cras[1], Corentin Cras-Méneur[1], Sara Kozma[2], George Thomas[2], Ernesto Bernal-Mizrachi[1]. *[1]Department of Internal Medicine, University of Michigan, MI, USA; [2]Department of Cancer and Cell Biology, University of Cincinnati, OH, USA.*

Diet has been demonstrated to be a key component of pancreatic β-cell programming during development, but the molecular mechanisms regulating this process are unclear. Numerous studies have demonstrated that low-caloric diets during pregnancy alter β-cell mass in the offspring and increase their odds of developing type 2 diabetes. Nutrients, growth factors and metabolites converge on the protein kinase mTOR (mammalian Target Of Rapamycin). mTOR signaling is critically involved in the regulation of growth and development of multiple tissue organs including the pancreas. Thus we hypothesize that mTOR plays a central role on β-cell programming during development.

To assess the role of mTOR signaling pathway in regulating the numbers of β-cell progenitors, mouse embryonic pancreatic rudiments (E13.0) were cultured with Rapamycin, a potent inhibitor of the mTOR kinase. Next, we investigated the role of mTOR signaling in β-cell development by generating genetically modified mice harboring conditional deletion of either mTOR or Tsc2 (a negative regulator of mTOR) in Pdx-1 progenitors cells. We then physiologically decreased mTOR signaling by exposing pregnant mice to a low-protein diet and assess the pancreatic effects in their offspring.

Our data showed a significant decrease in the number of pancreatic and endocrine progenitors (Pdx-1 and Ngn3 positive-cells respectively) in the Rapamycin-treated rudiments compared to controls. Rapamycin reduced the proliferative rate of Pdx-1 cells, suggesting that mTOR signaling is important for the expansion of pancreatic progenitor pool, a key determinant of final β-cell mass and pancreatic size. Conditional deletion of mTOR in pancreatic progenitors significantly reduced both β-cell mass and proliferation, whereas, deletion of Tsc2 had the opposite effect. Maternal low-protein diet during pregnancy results in offspring with near-normal body weight at birth, despite a shorter body length. Insulin content was also reduced compared to controls, suggesting that low-protein (as opposed to simply low-caloric) diet in dams during pregnancy may affect the number of insulin-producing β-cells in their offspring.

mTOR signaling is important in β-cell programming and development by controlling both the proliferation and differentiation of the endocrine progenitors.

PI-004

Mammalian Target of Rapamycin Regulates Placental Folate Transport. Fredrick J. Rosario, Theresa L. Powell, Thomas Jansson. *Center for Pregnancy and Newborn Research, Dept OB/GYN, University of Texas Health Science Center, San Antonio, TX, USA.*

Limited availability of methyl donors, such as folate, during pregnancy may result in abnormal gene methylation patterns and contribute to developmental programming. Fetal folate availability is critically dependent on maternal folate intake as well as placental transport. The molecular mechanisms regulating placental folate transport are unknown. Mammalian target of rapamycin (mTOR) is a protein kinase that controls cell growth and metabolism in response to nutrients and growth factors. mTOR exists in two complexes, mTORC1 and mTORC2. mTOR has been proposed to function as an important placental nutrient sensor and placental mTOR has

been shown to be down regulated in human IUGR. We tested the hypothesis that mTORC1 and mTORC2 signaling pathways regulate placental folate transport.

Human primary cytotrophoblast cells isolated from normal term placentas were cultured for 18 hrs and then transfected with siRNA targeting raptor (silences mTORC1), rictor (silences mTORC2) or scrambled siRNA (control). Cells were allowed to syncytialize in culture and at 90 hours ^{3}H-Methyltetrahydrofolate (MTHF) uptake was measured.

Transfection of cultured primary human trophoblast cells with raptor siRNA resulted in a 50% decrease in raptor protein expression and a marked inhibition in mTORC1 function as measured as the expression of phospho-Thr-389-s6K. Furthermore, transfection with rictor siRNA caused a 55% knock down of rictor protein levels and inhibition of mTORC2 function as assessed by phospho-Ser473-Akt expression. Silencing raptor or rictor did not affect syncytialization and did not induce apoptosis. Raptor silencing markedly decreased MTHF uptake in cultured primary human trophoblast cells (-58%, ANOVA, p=0.01, n=3) compared to control cells. Similarly, silencing of rictor inhibited MTHF transport (-57%, ANOVA, p=0.01, n=3).

Our data indicates that both mTORC1 and mTORC2 signaling regulates MTHF transport in primary human trophoblast cells. This is the first report of mTOR regulating folate transport, in any cell type. We speculate that regulation of placental folate transport by mTOR may alter the availability of methyl donors in the fetus, thereby providing a direct link between placental function, gene methylation and fetal programming.

PI-005

Withdrawn by Author

PI-006

Cardiovascular Disease in (Great)-Grandparents of Children Born with a Congenital Heart Disease: A Transgenerational Effect. Kim P.J. Wijnands[1], Sylvia A. Obermann-Borst[1], Eric J.G. Sijbrands[2], Mark F. Wildhagen[1,3], Willem A. Helbing[4], Régine P.M. Steegers-Theunissen[1,5]. *[1]Obstetrics and Gynecology, Erasmus Medical Centre, Rotterdam, Netherlands; [2]Internal Medicine, Erasmus Medical Centre, Rotterdam, Netherlands; [3]Urology, Erasmus Medical Centre, Rotterdam, Netherlands; [4]Pediatric Cardiology, Erasmus Medical Centre, Rotterdam, Netherlands; [5]Epidemiology & Clinical Genetics, Erasmus Medical Centre, Rotterdam, Netherlands.*

Hyperglycemia, dyslipidemia and hyperhomocysteinemia are features of an unhealthy lifestyle and are associated with aging diseases, in particular cardiovascular disease (CVD). During pregnancy these features in mothers also enhance the risk of having offspring with a congenital heart disease (CHD). We hypothesize that the prevalence of CVD is higher in (great)-grandparents of children with CHD due to unhealthy lifestyles, which they share with their children, being the mothers and fathers of these children. Therefore, we investigated associations between CVD in (great)-grandparents and modification by maternal factors, and the risk of having a grandchild with CHD.

A case-control family study has been conducted at the Department of Obstetrics and Gynecology at the Erasmus University Medical Centre in Rotterdam, The Netherlands. Through questionnaires detailed information on aging diseases and lifestyle was obtained from 379 families with and 427 families without a child with CHD.

With a response rate of 68% we found that the grandparents of a child with CHD reported significantly more often CVD compared to the control grandparents (17% versus 13%, p-value 0.034). We also found that maternal grandfathers with CHD offspring are treated more often for aging diseases (72% versus 63%, p-value 0.038). Furthermore, the fathers of the maternal grandfathers (i.e. great-grand fathers, showed more frequently CVD (44% versus 32%, p-value 0.015)). Moreover, CHD risk is higher (OR 1.7 (95%CI 1.2-2.5)) when one of the grandparents has CVD, even after adjustment for maternal age at conception, educational level, periconceptional B-vitamin use, hyperhomocysteinemia and hypercholesterolemia (OR 1.6 (95%CI 1.04-2.4)). A significant trend was shown for the number of affected grandparents (*P*-trend 0.002).

Aging diseases, especially CVD, in (great)-grandparents are associated with an increased risk of having a grandchild with CHD. Derangements of epigenetic programming of germ cells by unhealthy lifestyles may explain this first transgenerational effect.

PI-007

Reconstructing Diagnoses of Maternal Pregnancy Disorders for People Born 70 Years Ago – The Helsinki 1934-1944 Birth Cohort Study. Maiju Riihola[1], Samuel Sandboge[1], Clive Osmond[2], David J.P. Barker[2,3], Johan G. Eriksson[1,4], Eero Kajantie[1,4,5]. *[1]Institute for Health and Welfare, Finland; [2]MRC Lifecourse Epidemiology Unit, United Kingdom; [3]Oregon Health and Science University, USA; [4]Department of General Practice and Primary Health Care, University of Helsinki, Finland; [5]Children's Hospital, University of Helsinki, Finland.*

Most lifecourse studies in older adults have used surrogate markers of intrauterine conditions such as birth weight. Only a handful of such studies have diagnoses of maternal pregnancy disorders based on modern criteria. Our aims were: first, to reconstruct diagnoses of maternal hypertensive pregnancy disorders from maternity clinic and birth hospital records in 1934-44 and second, to assess whether these diagnoses predict diabetes in the adult offspring.

The Helsinki Birth Cohort Study comprises 13345 singletons born in Helsinki, Finland, during 1934-44, when maternity clinics were being introduced to combat challenges of maternal health including "nephrogestosis" and its most severe manifestation, eclampsia, which complicated 0.6% of pregnancies. Blood pressure and proteinuria, current cornerstones of diagnoses, were then not included in diagnostic criteria, although they were recognized as a part of the disorders and recorded. As adult outcome we studied diabetes, assessed by purchase of medication data of the National Social Insurance Institution, available from 1995 to 2002. Subjects dead or migrated before 1995 were excluded.

Adequate blood pressure (mean n=2.0; SD 1.6) and urinary protein (n=2.5; SD 2.0) measurements were available for mothers of 6410 newborns. Of them, 284 (4.4%) had pre-eclampsia (blood pressure 140/90 mmHg and semiquantitative urinary protein 1 mg/l); 1592 (24.8%) had gestational hypertension (blood pressure but no proteinuria). The adult offspring of mothers with gestational hypertension were more likely to have diabetes than the offspring of controls (11.0% vs. 8.4%; OR 1.39, 95% CI 1.09 to 1.71, adjusted for sex, year of birth, mother's BMI, father's occupational status, parity, birth weight and length of gestation). For offspring of mothers with pre-eclampsia, the OR was 1.28 (0.79 to 2.06).

We could reconstruct diagnoses of maternal hypertensive disorders for almost half of the subjects born 1934 to 1944. Adult offspring of mothers with gestational hypertension are at increased risk of diabetes.

PI-008

Intake of Methyl Donors during Pregnancy and Child Cognition at Age 7. Caroline E. Boeke[1], Matthew W. Gillman[2], Sheryl L. Rifas-Shiman[2], Eduardo Villamor[1,3], Lauren B. Guthrie[2], Emily Oken[2]. *[1]Epidemiology and Nutrition, Harvard School of Public Health, USA; [2]Population Medicine, Harvard Pilgrim Health Care Institute and Harvard Medical School, USA; [3]Epidemiology and Environmental Health Sciences, University of Michigan School of Public Health, USA.*

Animal models indicate that deficiency in choline and other methyl donor nutrients in utero can impair offspring cognition, especially memory, through epigenetic mechanisms. We investigated the extent to which methyl donor nutrient intake in a healthy population of pregnant women is related to child cognition.

Among 898 mothers in the pre-birth cohort Project Viva, using FFQs and nutritional supplement interviews we estimated maternal dietary intakes of vitamin B12, betaine, choline, and folate periconceptionally and during the 2nd trimester. Using multivariable linear regression, we examined associations of these nutrients with offspring memory at age seven years as measured by the Wide Range Assessment of Memory Learning (WRAML) and verbal and nonverbal intelligence using the Kaufman Brief Intelligence Test (KBIT-2).

Mean daily maternal periconceptional nutrient intakes were (mean [SD]) 11.2 (15.9) mcg vitamin B12, 249 (109) mg betaine, 336 (64) mg choline, and 796 (437) mcg folate. Mean age seven cognitive test scores were 17.1

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(4.4) points on the WRAML, 113.8 (14.1) on the verbal KBIT, and 107.6 (16.6) on the nonverbal KBIT. In a model adjusted for maternal intake of other methyl donors, age, race/ethnicity, education, KBIT score, parity, smoking, and fish and energy intake during pregnancy, paternal education, HOME score, and child age, sex, and primary language, for every 65 mg/day (~1 SD) increment of periconceptional choline intake, WRAML test score was 0.3 points higher (95% CI: 0.01, 0.6), and for every 440 mcg/day (~1 SD) of periconceptional folate intake, WRAML was 0.3 points higher (95% CI: 0.00, 0.7). Neither periconceptional intake of the other nutrients, 2nd trimester intake of any of the nutrients, nor intakes of methionine, vitamin B6, iron, or zinc, was associated with the outcomes.
Higher maternal periconceptional intakes of choline and folate were associated with improved child memory at age 7.

PI-009

Breastfeeding Duration Is Associated with Food Preferences in Girls and Feeding Behavior in Boys at 4 Years of Age. Marilyn Agranonik[2,4], André Krumel Portella[2], Michel J. Meaney[1], Robert D. Levitan[3], Patrícia Pelufo Silveira[1,2]. *[1]Douglas Mental Health University Institute, Montreal, QC, Canada; [2]Núcleo de Estudos da Saúde da Criança e do Adolescente – Hospital de Clínicas de Porto Alegre – Universidade Federal do Rio Grande do Sul, Porto Alegre/RS, Brazil; [3]Centre for Addiction and Mental Health and Department of Psychiatry, University of Toronto, Toronto, Canada; [4]Universidade do Vale do Rio dos Sinos, Sao Leopoldo /RS, Brazil.*
Breastfeeding confers protection against several chronic diseases later in life, such as obesity and related complications. However, little is known about the impact of its duration on food preferences and feeding behavior during infancy. In the current study we examined the relationship between breastfeeding duration (BD) and 1) food preferences at four years of age using a food frequency questionnaire and 2) feeding behavior (FB) using the Child Eating Behavior Questionnaire (CEBQ).
Seventy-one children participating in a longitudinal study of Maternal Adversity, Vulnerability and Neurodevelopment (MAVAN) were enrolled. Pearson correlation coefficient was used to evaluate the relationship between total or exclusive BD with percentage of macronutrients consumption and FB (Satiety, Slowness, Fussiness, Food responsiveness, Enjoyment of food, Desire to drink, Emotional overeating and Emotional undereating).
Girls who had longer BD showed an increased percentage of protein consumption in their habitual diets at four years ($r=0.40$ $p= 0.024$, for total BD and $r=0.37$ $p=0.032$, for exclusive BD). This correlation was not significant in boys. However, amongst boys but not in girls, BD was negatively associated with food responsiveness ($r= -0.44$, $p=0.010$ for total BD and $r= -0.42$, $p=0.016$ for exclusive BD) and emotional under-eating ($r= -0.35$, $p=0.048$ for total BD and $r= -0.35$, $p=0.046$ for exclusive BD).
Protein intake stimulates insulin and IGF-1 metabolism and consequently leads to cell proliferation, growth, and increased adipose tissue. Considering that breast milk has lower content of protein when compared to infant formulas, it is possible that the longer the BD, the later the physiological rebound adiposity, which may protect against later obesity and related diseases in girls. In boys, BD may protect against undesired feeding behaviors that may also affect healthy growth. In sum, these findings suggest a gender specific effect of BD, influencing food preferences in girls and FB in boys.

PI-010

Increased Risk for Affective Disorders Programmed In Utero? High Prenatal Maternal Cortisol Concentrations and Volumes of the Amygdala and Hippocampus in the Offspring at 6-9 Years of Age. Claudia Buss[1], Elysia P. Davis[2], Jens C. Pruessner[3], Tugan L. Muftluer[2], Kevin Head[2], Anton Hasso[2], Curt A. Sandman[2]. *[1]Pediatrics, University of California, Irvine, USA; [2]Psychiatry and Human Behavior, University of California, Irvine, USA; [3]Psychiatry, Psychology, Neurology and Neurosurgery, McGill University, Canada.*
Because fetal brain development proceeds at an extremely rapid pace, early life experiences have the potential to alter the trajectory of neurodevelopment. Alterations especially in limbic structures have been associated with a range of neuropsychiatric disorders, including affective disorders. Studies in non-human primates and rodents have shown that such alterations can be induced in the offspring of mothers by prenatal exposure to exogenous glucocorticoids or chronic stress. To this date, the association between exposure to prenatal maternal cortisol concentrations and size of limbic structures has not been studied in human subjects.
In the current prospective longitudinal study we included women for whom serial data on cortisol concentrations were available at five time points over the course of gestation. When the offspring from the target pregnancy were between six to nine years of age, volumes of the hippocampus and amygdala were assessed by manual segmentation of T1 magnetic resonance (MR) images, acquired by a Phillips 3T Tesla.
After controlling for potentially confounding postnatal factors, high maternal cortisol concentrations were associated with larger amygdala volumes in the 6-9 year old offspring. Furthermore, high maternal prenatal cortisol concentrations were associated with higher anxiety levels in their offspring. Analyses stratified by sex suggested that these associations were significant in female but not in male offspring. Prenatal maternal cortisol concentrations were not associated with hippocampal volumes.
These findings are in line with studies in rodents and non-human primates and suggest that higher maternal cortisol concentrations during pregnancy are associated with changes in limbic structures, which may increase the offspring's susceptibility for neuropsychiatric disorders.

PI-011

Adrenal and Thyroid Hormones, and Behavioral Traits in the Programming Model by Early Weaning. Juliana G. Franco[1], Egberto G. Moura[1], Natália S. Lima[1], Alex C. Manhães[2], Mabel C. Fraga[2], Magna C.F. Passos[1], Elaine Oliveira[1], Patrícia C. Lisboa[1]. *[1]Laboratory of Endocrine Physiology, State University of Rio de Janeiro, Brazil; [2]Laboratory of Neurophysiology, State University of Rio de Janeiro, Brazil.*
Thyroid and adrenal function can be programmed by nutritional and hormonal factors. Pharmacological inhibition of lactation with bromocriptine (a PRL inhibitor) leads to overweight, hypothyroidism and higher adrenal hormones in adult offspring. Recently, we have shown that early weaning (EW) with no use of pharmacological substances or maternal separation programmed for obesity, hyperleptinemia, and leptin and insulin resistance at adulthood. Here, using this same model, we studied the adrenal and thyroid function of neonate and adult offspring, hormones that influence behavior.
After birth, lactating rats were separated in: EW (early weaning) - dams were wrapped with a bandage to interrupt lactation during the last three days of lactation, and C (control) - dams whose pups had free access to milk throughout lactation (21 days). Total catecholamines were quantified by the trihydroxyindole method and serum thyroids hormones and corticosterone were measured by RIA. From PN160 to PN178, EW and C offspring were submitted to behavioral tests: elevated plus-maze (EPM); hole board arena (HB) and radial arm water maze (RAWM).
As expected, EW pups had lower body weight and length at postnatal day (PN) 21, while they showed higher food intake, body weight and length at PN 180. Adrenal evaluations were normal, but serum T3 and TSH were lower in neonate EW (-55% and -44%, respectively). By the contrary, adult EW offspring presented higher adrenal catecholamine content (total: +31%, relative: +54%) but no changes regarding serum corticosterone and thyroid status. Behavioral evaluation showed that the EW group was programmed for higher motor activity and lower risk assessment.
Thus, lactation interruption with breast banding causes hypothyroidism that is restored at adulthood. The main consequence of the programming model by EW is the adrenal medullary dysfunction with consequent behavioral alterations.

PI-012

Rho Kinase Mediates the Basal Pressor Response, but Not the Enhanced Pressor Response to Acute Angiotensin II in Intrauterine Growth Restricted Rats. Norma B. Ojeda[1], Thomas P. Royals[2], Barbara T. Alexander[2]. *[1]Pediatrics, University of Mississippi Medical Center, MS, USA; [2]Physiology, University of Mississippi Medical Center, MS, USA.*
Placental insufficiency in the rat programs hypertension and an enhanced renal vascular responsiveness to acute angiotensin II (ANG II) in male intrauterine growth restricted (IUGR) rats. Renal constrictor responses to ANG II are linked to hypertension suggesting involvement of the angiotensin type 1 receptor (AT_1R) and its downstream signaling pathways. Therefore,

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we hypothesized that post-AT_1R signaling mediated via Rho kinase activation may contribute to the enhanced pressor response to acute ANG II observed in male IUGR rats.
Animals were pretreated with RAS blockade (250 mg/L enalapril for one week to normalize blood pressure) followed by determination of systemic hemodynamic parameters before (baseline) or after acute (30 min) ANG II (100 ng/kg/min) without or with inhibition of Rho kinase by fasudil (bolus of 1 mg/min, then 33 mg/kg/min), n=6 per group.
At baseline or under conditions of blockade of the endogenous renin angiotensin system, mean arterial pressure (MAP) did not differ upon comparison of IUGR to control (118± 3 vs. 118± 4 mmHg, respectively). As previously reported, the pressor response to acute ANG II was enhanced in male IUGR (176± 2 mmHg*†, increase of 58 mmHg above baseline) relative to male control (161± 3 mmHg*; increase of 43 mmHg above baseline). Inhibition of Rho kinase attenuated the pressor response to acute ANG II in IUGR (164± 5 mmHg*‡#; decrease of 12 mmHg) and control (144± 6 mmHg*‡; decrease of 17 mmHg) rats. However, the differential response to acute ANG II was not abolished by Rho kinase inhibition in IUGR (increase of 46 mmHg above baseline) compared to control (increase of 26 mmHg above baseline).
* $P < 0.05$ vs. Baseline; † $P < 0.05$ vs. Control ANG II; ‡ $P < 0.05$ vs. ANG II counterpart; # $P < 0.05$ vs. Control ANG II + fasudil
Thus, these data indicate that post-receptor signaling of the AT_1 receptor mediated via the Rho kinase signaling pathway does not contribute to the potentiated pressor response to acute ANG II programmed by placental insufficiency in male IUGR offspring. NIH HL074927, HL51971.

PI-013

Heart Disease Link to Prenatal Hypoxia and Oxidative Stress. B. J. Allison, E. A. Herrera, E. J. Camm, H. G. Richter, C. E. Blanco, A. D. Kane, F. B.P. Wooding, C. M. Cross, K. L. Brain, D. A. Giussani. *Physiology Development & Neuroscience, University of Cambridge, United Kingdom.*
The prenatal environment interacts with our genes to determine cardiovascular risk. However, mechanisms mediating developmental programming remain elusive, precluding the identification of potential clinical therapy. The most common complications in pregnancy are reductions in oxygen and nutrient delivery to fetus. Using an integrative approach at the isolated organ, cellular and molecular levels, we tested the novel hypothesis that oxidative stress in the fetal heart and circulation underlies the molecular basis via which prenatal hypoxia programmes cardiovascular disease in adulthood.
The hypothesis was tested in a longitudinal study by investigating the effects of maternal treatment of hypoxic (13% O2) pregnancy with vitamin C (5 mg/ml drinking water) on the cardiovascular system of the offspring at two stages of life: in the fetus at the end of gestation and at four months of adulthood. On day 6 of pregnancy, rats (n=20 per group) were exposed to normoxia or hypoxia ± vitamin C. Maternal food intake was unaffected. At day 20, tissues were collected from one male fetus per litter per group from one set of dams (n=10). The remaining 10 litters per group were allowed to deliver. At four months, tissues were either perfusion fixed, frozen, or dissected for isolated organ preparations from one male per litter per outcome variable. All data were evaluated by stringent statistical tests.
In the fetus, hypoxic pregnancy promoted aortic thickening with enhanced nitrotyrosine staining and an increase in the cardiac HSP70 expression (all $p<0.05$). At adulthood, offspring of hypoxic pregnancy had markedly impaired NO-dependent relaxation in femoral resistance arteries, and increased myocardial contractility associated with enhanced sympathetic but depressed parasympathetic cardiac reactivity (all $p<0.05$). Maternal vitamin C prevented these effects in fetal and adult offspring of hypoxic pregnancy.
Developmental hypoxia programmes cardiovascular disease secondary to oxidative stress. The study has broad scientific and clinical significance. The data offer insight into mechanism and targets for intervention against a fetal origin of cardiac and peripheral vascular disease in offspring of risky pregnancy.
Supported by the British Heart Foundation, BBSRC and The Royal Society.

PI-014

Adaptive Growth of Healthy Cardiomyocytes in Diseased Embryonic and Fetal Hearts. Kom V. Yin[1], Jonathan G. Bensley[1], Jörg D. Drenckhahn[2], Mary J. Black[1]. *[1]Anatomy and Developmental Biology, Monash University, Victoria, Australia; [2]Max-Delbrück Center for Molecular Medicine, Berlin, Germany.*
It is important to understand how the developing heart responds to insults in utero because cardiomyocytes cease proliferating soon after birth when they become terminally differentiated. Hence a reduced complement of cardiomyocytes at birth reduces the life-long functional reserve of cardiomyocytes. A mouse model exhibiting heart-specific inactivation of Hccs has been developed. Hccs is an X-linked gene encoding Holocytochrome C synthase which is essential in mitochondrial respiration. In heterozygous Hccs-knockout females, 50% of their cardiomyocytes are dysfunctional at mid-gestation due to the mitochondrial defect. Although their hearts are fully functional at birth, it is not known whether they are able to fully compensate for 50% of damaged cardiomyocytes in terms of cardiomyocyte number. Hence, this study aimed to examine how the mouse heart responds to damage to 50% of cardiomyocytes during mid-gestation.
At birth, cardiomyocyte number was estimated in Hccs-knockout and age-matched control hearts using an optical disector-fractionator approach, cardiomyocyte size was determined by measuring cardiomyocyte cross-sectional area whilst cardiomyocyte proliferation was detected by immunofluorescence staining for Ki-67.
At birth, body weight was not significantly different between the groups whilst absolute and relative heart weight and volume were significantly reduced in the knockouts. Importantly, the number of cardiomyocytes was significantly reduced in the knockout hearts when compared to controls at birth and this was accompanied by a significant increase in cardiomyocyte size. In addition, cardiomyocyte proliferation was significantly downregulated in knockout hearts when compared to controls at birth.
Disease in 50% of cardiomyocytes at mid-gestation is associated with decreased proliferation, a decreased number of cardiomyocytes and compensatory cardiomyocyte hypertrophy at birth. This cardiomyocyte deficit may adversely impact on postnatal cardiac function.

PI-015

Cardiomyocyte Growth and Maturation during Mid to Late Gestation and the Effect of Preterm Birth. Jonathan G. Bensley[1], Lynette Moore[2], Robert De Matteo[1], Richard Harding[1], Mary J. Black[1]. *[1]Department of Anatomy and Developmental Biology, Monash University, Victoria, Australia; [2]Department of Histopathology, Women's and Children's Hospital, South Australia, Australia.*
Preterm birth affects 8-12% of all pregnancies and is the leading cause of neonatal morbidity and mortality. We have previously shown that moderate preterm birth in lambs results in cardiomyocyte hypertrophy, derangements of cardiomyocyte maturation and increased interstitial collagen deposition. Our present aim was to examine in autopsied tissue the effect of preterm birth on cardiomyocyte size, maturation and ploidy and levels of interstitial collagen in the heart of the preterm human infant.
Archived heart tissue obtained at perinatal and neonatal autopsy was analysed. We established the normal growth and maturation parameters of human cardiomyocytes using gestational controls (*fetuses that had died acutely in utero*) (n=37) and compared these hearts to neonates who died following preterm birth (n=30). Specifically, we used confocal microscopy to assess cardiomyocyte volume, ploidy and maturation. Immunohistochemistry was used to analyse the population of cardiac progenitor cells and cardiomyocyte proliferation. Image analysis of picrosirius red-stained sections was used to quantify collagen deposition.
There was a wide variation in cardiomyocyte size in both the preterm hearts and gestational controls. Cardiomyocyte size and maturation was unaffected by preterm birth. Importantly, there was a marked reduction in the number of proliferating cardiomyocytes in the hearts of the preterm infants, within 12 hours of preterm birth. This was coupled with a reduction in the number of cardiac (c-kit+/Lin-) progenitor cells.
There was no effect of preterm birth on cardiomyocyte size, maturation or ploidy, however, the rate of cardiomyocyte proliferation was markedly reduced. This is likely to lead to a reduction in the complement of cardiomyocytes in the s of infants born preterm. A reduced number of

cardiomyocytes and cardiac progenitor cells at the beginning of life in the preterm infant are likely to compromise both the functional reserve and adaptive capabilities of the heart in adulthood and thus predispose for the development of cardiovascular disease.

PI-016

***In-Utero* and Postnatal Exposure to a High Fat Nutritional Environment Alters Gene Expression Patterns of Clock and Clock-Controlled Genes in Murine Hearts.** Aaron J. Stokes, Kimberley D. Bruce, Kerry L. Hyde, Mark A. Hanson, Christopher D. Byrne, Felino R. Cagampang. *Institute of Developmental Sciences, Academic Unit of Human Development and Health, University of Southampton Faculty of Medicine, Southampton, United Kingdom.*

The prevalence of cardiovascular diseases (CVD) is increasing at an alarming rate. It has been shown that suboptimal *in-utero* and postnatal nutritional environments can increase the offspring's susceptibility to CVD in adulthood. Emerging evidence demonstrates the role of the circadian clock system in the pathogenesis of CVD. In this study, we examined whether *in-utero* and postnatal exposure to high fat (HF) nutritional environment can alter expression pattern of clock and clock-controlled genes in the murine adult offspring heart.

Female C57/BL6J mice were fed either a HF or control chow (C) diet pre-conception and throughout pregnancy and lactation. Weaned offspring were fed the HF or C diet, generating the offspring groups: C/C, C/HF, HF/C, HF/HF. Whole hearts were taken from 15-week old male offspring killed at six time points over a 24h light-dark period (n=3-5 hearts per group). We determined gene transcript levels for the clock genes, *CLOCK* and *PER2*, and clock-controlled genes, *PAI-1 and SIRT1*, using RT-PCR. Statistical significance between groups was calculated by ANOVA. Cosine wave analysis (Acro program) was used to compute the acrophase (time of peak) of the 24h rhythm in gene expression.

Expression levels for *CLOCK* was found to be 1.5 ($p<0.001$) and 1.4 fold higher in HF/C and HF/HF groups, respectively, vs. C/C. *PAI-1* levels were 2.3 fold higher ($p<0.001$) in HF/HF vs. C/C, and for *PER2* this was 1.6 fold higher ($p<0.05$) in HF/C vs. C/C. No differences in expression levels were observed for *SIRT1* between treatment groups. Cosine wave analysis showed that pre- and post-natal exposure to HF diet resulted in phase-shifting in peak expression of *CLOCK*, *PER2* and *PAI-1* genes. *SIRT1* also showed a phase shift in peak expression but only in the C/HF group, suggesting that prenatal HF exposure may prevent the phase shift brought about by post-weaning HF feeding.

The results suggest that rhythmic expression of clock and clock-controlled genes are disrupted following early life exposure to maternal HF nutrition, and could also be further modified by post-natal HF feeding. These changes may have deleterious effects on cardiovascular function, increasing cardiovascular risk in adulthood.

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PI-017

Femoral Vascular Responses Evoked by Different Patterns of Sympathetic Nerve Stimulation in Developmentally Programmed Rats. William Rook, Andrew M. Coney, Janice M. Marshall. *CEM-Physiology, University of Birmingham, West Midlands, United Kingdom.*

Chronic hypoxia in utero (CHU) is used as a developmental programming stimulus and is associated with differences in cardiorespiratory function (Fowden, 2006). In the chronically hypoxic chick embryo, there is sympathetic hyperinnervation of the femoral artery in the d19 fetus (Rouwet, 2002) and exaggerated vasoconstriction at 14-15 weeks old (Ruijtenbeek, 2003). In our accompanying abstract, we show that in adult CHU rats there is increased sympathetic nerve density in the blood vessels supplying muscle in the hindlimb, and greater basal muscle sympathetic nerve activity than in normal (N) rats. In N rats, we have demonstrated that neuropeptide Y (NPY) can partly mediate sympathetically evoked vasoconstriction in muscle (Coney, 2007). We have now investigated whether these sympathetic nerve changes lead to alterations in the vasoconstrictor responses evoked by sympathetic nerve stimulation.

Acute experiments were performed on male CHU rats that were the offspring of pregnant Wistar dams exposed to 12% O_2 from day 10-20 of gestation (Coney, 2010) and on age-matched (10-12wk old) N rats. Vascular responses to 1min of lumbar sympathetic chain stimulation were recorded. Stimulation comprised 120 pulses of 1ms duration in three different patterns; 2Hz continuous, bursts of 20 pulses repeated every 10sec at 20Hz and 40Hz. Responses were recorded before and during infusion of the NPY antagonist BIBP3226 ($10\mu g.kg^{-1}.min^{-1}$).

Femoral vascular resistance (FVR) increased in both N and CHU rats indicating vasoconstriction However, peak FVR during stimulation was significantly lower in CHU rats relative to N rats at 2Hz ($p=0.0198$), 20Hz ($p=0.0392$) and tended to be lower at 40Hz ($p=0.09$). BIBP3226 did not significantly reduce the overall integrated FVR response in CHU rats, but did reduce the vasoconstrictor responses in N rats.

These results indicate that, despite increased density of sympathetic innervation as well as increased ongoing nerve activity, the vasoconstrictor response to sympathetic activation is smaller in CHU rats. Additionally, in N rats neuropeptide Y modulates the sympathetically-mediated vasoconstriction, however, we find no evidence of such modulation in CHU rats. Thus, there appear to be adaptations in CHU rats in sympathetic transmission controlling hindlimb vascular tone.

PI-018

Brain Derived Neurotrophic Factor Levels in Preeclampsia. Vandita D'Souza[1], Anitha Kilari[1], Savita Mehendale[2], Hemlata Pisal[1], Sadhana Joshi[1]. *[1]Nutritional Medicine, Interactive Research School for Health Affairs, Maharashatra, India; [2]Obstetrics and Gynecology, Bharati Medical College and Hospital, Maharashatra, India.*

Preeclampsia (PE) is a unique hypertensive disorder in pregnancy leading to maternal and fetal morbidity. Our earlier studies in PE suggest a causal relationship between altered angiogenic factors and birth outcome. Recent studies suggest that brain derived neurotrophic factor (BDNF) can stimulate angiogenesis. The present study for the first time compares the levels of maternal and cord BDNF from preeclamptic and normtensive women.

Preeclamptic women delivering at term, FT-PE (n=65), preeclamptic women delivering preterm, PT-PE (n=51) and normotensive women, NC (n=90) (control) were recruited for the study from Bharati Hospital Pune, India. BDNF levels were measured in both mother and cord blood plasma using the BDNF Emax Immuno Assay System Promega kit.

Maternal plasma BDNF levels were lower ($p = 0.027$) in the FT-PE group (342.2 ± 138.7 pg/ml) as compared to the NC (403.9 ± 207.7 pg/ml) group. Similarly, levels of BDNF were reduced in the PT-PE group (291.0 ± 128.7 pg/ml) as compared to both NC ($p = 0.001$) and FT-PE ($p = 0.047$) groups. Cord plasma BDNF levels were higher ($p = 0.001$) in the FT-PE group (1077.9 ± 647.5 pg/ml) as compared to the NC (730.2 ± 605.2 pg/ml) group. BDNF levels were positively correlated with duration of gestation ($r = 0.194$, $p = 0.009$, $df = 180$); negatively with placental weight ($r = - 0.199$, $p = 0.029$, $df = 118$) and diastolic blood pressure ($r = - 0.197$, $p = 0.008$, $df = 179$).

Differential regulation of cord BDNF levels in preterm PE as compared to term PE may be associated with severity of the disease and gestation suggesting that term and preterm PE may be separate disease entities. The possible mechanisms underlying the association between angiogenesis and neurotrophins in PE are unclear and future studies need to address this issue. It is also important to follow up babies born to mothers with PE for cardiovascular risk or cognitive impairment in later life since they may be at risk for neurodevelopmental disorders.

PI-019

Maternal Diet-Induced Obesity in C57BL/6 Mice Alters Vascular Function in Their Offspring but Not Metabolism or Vascular Remodelling after Intravascular Injury. Rachel S. Dakin, Patrick W.F. Hadoke, Brian R. Walker, Jonathan R. Seckl, Amanda J. Drake. *Department of Endocrinology, Centre for Cardiovascular Science, University of Edinburgh, United Kingdom.*

The global increase in obesity means an increasing number of babies are born to obese mothers. Whilst exposure to an obese environment in utero is a risk factor for cardiovascular disease in later life, the extent to which both cardiovascular risk factors and vascular lesion development can be attributed to early life programming is uncertain.

Using a mouse model we investigated the hypothesis that maternal obesity causes adverse metabolic changes, alters vascular function and increases lesion formation following vascular injury in the offspring.

C57Bl/6 female mice maintained on obesogenic or control diet from five weeks were mated at 15 weeks (CON 22.6g ±0.5, DIO 31.9g ± 1.6), remaining on experimental diet during pregnancy and suckling. Litters were reduced to five pups and offspring (n=6-9) of obese (DIO) and control (CON) mothers were weaned onto standard diet. At 12 weeks glucose tolerance and tail cuff blood pressure (BP) were measured and femoral arteries removed for functional analysis. A second cohort was killed at 19 weeks, four weeks after femoral artery injury; femoral arteries were excised and vascular remodelling assessed by histology.

Physical and metabolic parameters were similar in both groups except that plasma triglycerides were lower ($p<0.05$) in DIO. Similarly, neither BP nor neointimal lesion size were altered in the DIO group. There were, however, changes in vascular function: the dose response curve to phenylephrine (but not to serotonin) was shifted to the right in DIO, an effect that was statistically significant after removal of the endothelium (pD2 DIO 5.67 ± 0.92, CON 6.52 ± 0.36; $p<0.05$). Furthermore, sensitivity to acetylcholine (-log(EC50) DIO 6.68 ± 0.49, CON 7.56 ± 0.33; $p<0.001$) was also reduced in intact DIO arteries, whereas endothelium-independent relaxation (sodium nitroprusside) was unaltered.

These results indicate that exposure to an obese environment in utero can programme alterations in the function of vascular smooth muscle and endothelial cells in adult offspring. These changes are not associated with overt alterations in metabolic function nor predict increased vascular lesion formation.

PI-020

Maternal Protein Restriction Leads to Bronchial Hyper-Responsiveness in Rat Male Offspring. Shelley A. Davis[1], Elin R. Thomas[2], John W. Holloway[1], Christopher Torrens[2]. *[1]Infection, Inflammation & Immunity, University of Southampton, United Kingdom; [2]Human Development & Health, University of Southampton, United Kingdom.*

Maternal nutrition is now recognized to play a role in the development of cardiovascular disease in the offspring; however the influence on subsequent respiratory disease is still unclear. Low birth weight infants have greater risk of developing COPD in adulthood (Barker *et al.*., 1991), while as children they demonstrate increased airway responsiveness (Chan *et al.*., 1988). Since the primary cause of morbidity and mortality in asthma patients is this hyper-responsive bronchial smooth muscle we investigated the effect of maternal protein restriction on offspring bronchial responsiveness.

Pregnant Wistar rats fed either control (C, 18% casein) or protein restricted (PR, 9% casein) diet from conception to term, before being returned to standard chow immediately postpartum. At ~150 days of age male offspring were sacrificed by cervical dislocation and lung tissue was harvested. Segments of bronchi were dissection from the left lobe of the lung and mounted on a wire myograph. Cumulative responses to carbachol (CCh, 0.1 nM – 100 μM), angiotensin II (Ang II, 1 pM – 1 μM) and the thromboxane mimetic U46619 (1 pM – 1 μM). Data are mean ± SEM and differences assessed by Student's t test. Significance accepted if $p<0.05$.

Both CCh and U46619 produced a concentration-dependent bronchoconstrictions that were significantly enhanced in the PR group compared (CCh: C, 0.79 ± 0.02 g, n=6; PR, 1.17 ± 0.03 g, n=4; $p<0.001$; U46619: C, 0.60 ± 0.02 g, n=4; PR, 0.79 ± 0.02 g, n=4; $p<0.001$). Responses to Ang II were very modest and did not differ between the groups.

This study demonstrates that the bronchi from male offspring of protein restricted dams demonstrate a significantly enhanced constriction to bronchoconstrictors. This suggests that poor maternal nutrition may predispose offspring to a phenotype similar to that of asthma.

PI-021

Systemic Artery Biochemical and Biomechanical Changes in Ovine Model of Intrauterine Growth Restriction. Reuben B. Dodson[1], Carson C. Petrash[1], Paul J. Rozance[2], Kendall S. Hunter[3], Virginia L. Ferguson[1]. *[1]Mechanical Engineering, University of Colorado at Boulder, CO, USA; [2]Pediatrics and Neonatology, University of Colorado at Denver Anschutz Medical Campus, CO, USA; [3]Bioengineering, University of Colorado at Denver Anschutz Medical Campus, CO, USA.*

Human and experimental intrauterine growth restriction (IUGR) results in increased placental blood flow resistance and fetal arterial pressure and pulsatility, items shown to cause vascular remodeling in adolescents and adults. However, consequences of such changes in the fetus are understudied and may link IUGR to adult hypertension. We hypothesize that IUGR causes decreased systemic fetal artery compliance (or increased stiffness) due to altered extracellular matrix (ECM) composition and structure.

An ovine model of placental insufficiency-induced IUGR, via exposure of the pregnant ewe to elevated ambient temperatures, was used. Umbilical and carotid arteries were harvested from near term fetuses and tested using pressure-diameter inflation to compare passive compliance in control (CON) (n=6 & n=4, respectively) and IUGR (n=3 & n=2). Arterial compliance data was examined for collagen engagement (transition stretch), diameter stretch, and stiffness at mean physiologic blood pressure for fetal sheep (44.5 mmHg). ECM composition was measured in CON and IUGR arteries via biochemical assays.

IUGR reduced fetal weight by 32% (3.20±0.15 vs. 2.17±0.37 kg, $p<0.05$) and placental weight by 42% (455±44 vs. 263±16 g, $p<0.05$). The umbilical artery was less compliant in the IUGR vessels (292±27 vs. 870±308 mmHg, $p<0.05$) while the transition (1.33±0.08 vs. 1.10±0.02 mm/mm, $p<0.10$) and diameter stretch (1.43±0.07 vs. 1.23±0.05 mm/mm, $p<0.15$) trended towards significance. The ECM assay showed decreased elastin in IUGR umbilical vessels (70.2±7.3 vs. 40.7±1.0 μg/mg, $p<0.05$). The IUGR carotid artery showed decreased compliance (281±35 vs. 562±19 mmHg, $p<0.05$) and elastin content (61.5±6.9 vs. 38.6±10.4 μg/mg, $p<0.15$).

At physiological stresses, arterial compliance decreased in IUGR, where increased modulus and decreased transition stretch indicates less elastin and increased collagen engagement. Elastin deposition in arteries is highest in utero and is generally stable for life. Because vessel stiffness with reduced fetal weight has been a great predictor for cardiovascular disease, elastin deposition may provide a potential link between IUGR and adult hypertension.

PI-022

Life Course Risk Factors for Adult Pre-Hypertension and Hypertension. Alexandre A. Ferraro[1], Viviane C. Cardoso[2], Carlos Grandi[3], Ricardo C. Cavalli[2], Antônio Augusto M. da Silva[4], Heloísa Bettiol[2], Marco C. Barbieri[2]. *[1]Faculdade de Medicina da Universidade de SP - Campus Capital, Brazil; [2]Faculdade de Medicina da Universidade de SP - Ribeirão Preto, Brazil; [3]Maternidade Sardá, Argentina; [4]Universidade Federal do Maranhão, Brazil.*

It has been demonstrated in the last years that chronic diseases (CD) are associated not only with adult risk factors (RF), but also with predictors that occurred decades before the onset of disease. The pathway through which these early RF influence adult outcomes is not clear. Among the CD hypertension (HT) is the most prevalent one. Recently it has been suggested that systolic pressure between 120-140 of mm Hg and a diastolic pressure between 80-90 mm Hg - pre-hypertension (PHT) - are linked to a higher health risk. The objective of this study is to assess the independent association of early life and adult risk factors for PHT and HT.

A prospective cohort of all living born of the city of Ribeirao Preto, Brazil, was assessed at birth (1978/79), school-age (1987/88) and adulthood (2002/04). Data on neonatal variables, socioeconomic position and anthropometry of all three moments as well as adult RF for HT were present for 1143 of the 6484 eligible subjects. Conditional weight analysis was performed to assess the risk of repeated-in-time measurements.

The lower the ponderal index (PI) at birth the higher the prevalence of PHT and HT in adulthood. Adult RF for HT were associated with the outcome in the univariate analysis, but in the adjusted analysis PI at birth was more significantly linked with adult diastolic pressure (lower PI RR=2,06 for PHT and RR=4,72 for HT).

early life nutrition status has an important role in predicting adult diastolic PHT and HT than adult RF.

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PI-023

National Children's Study Vanguard Pilot Study: Recruitment Experiences in the Orange County, CA (OCCA) Vanguard Center. Dean Baker[1], James Swanson[1]. [1]*Medicine, UC Irvine, CA, USA;* [2]*Pediatrics, UC Irvine, CA, USA.*

The Vanguard Center pilot study used household-based recruitment in geographically defined neighborhoods (called segments) randomly sampled within the NCS study locations (mostly counties). Our aim is to evaluate the recruitment methods and yield after 2 yrs of work.

Over 25,000 census blocks were aggregated into contiguous clusters of 1850 potential segments, each with an estimated 24.8 births/yr. 15 segments were randomly selected, and they had 10,502 dwelling units (DU). Staff attempted to identify all age-eligible (18 to 49 years) women in the dwelling units by conducting in-person household interviews. Women in the first trimester of pregnancy were asked to enroll immediately; non-pregnant women were followed by telephone contact to assess changes in pregnancy status and likelihood of becoming pregnant.

89% of DUs were enumerated (completed a household census). 5153 age-eligible women were identified, and 92% completed pregnancy screening interviews, identifying 128 pregnant and 3480 non-pregnant women were identified. Non-pregnant women were followed by telephone contact, but only 45.6% completed this protocol (18% had invalid or wrong numbers, 2% were secondary refusals, and 34.2% reached maximum number of contacts allowed). Over 1-year, 112 (68%) of the pregnant-eligible women enrolled, and 88 (79%) were followed to the birth. Overall recruitment and retention rate was 44%.

The 44% rate was substantially less than the 68% target but was generally consistent with experiences of other community-based studies. This indicates the necessity of devoting substantial resources to community surveillance (monitoring for dwelling unit turnover) and to increase the intensity of follow-up of non-pregnant women. The Vanguard protocol did not allow for the use of paid media and provided a limited community outreach and engagement effort (2 full time equivalent staff for the overall county of more than 3.5 million residents and 15 dispersed, diverse segments). Consent rates possibly could be increased by providing greater resources for media and publicity, community engagement, and enhancing partnerships with medical care providers who could encourage study participation. These strategies are currently being evaluated in new NCS recruitment pilots. cost of population recruitment and enrollment is a high, but it represents a very small proportion of the overall cost of the NCS.

PI-024

Lessons Learned in the Vanguard Phases of the US National Children's Study. James M. Swanson[1], Dean Baker[1], Kjersti Aagaard[3], Pathik Wadhwa[1], Faustman Elaine[4], Hirschfeld Steve[2]. [1]*Pediatrics, UC Irvine, CA, USA;* [2]*National Children's Study, NICHD-NIH, MD, USA;* [3]*OG/GYN, Baylor College of Medicine, TX, USA;* [4]*Public Health, University of Washington, WA, USA.*

The National Childrens Study (NCS) was designed to be a birth cohort of 100,000. Seven Vanguard Centers performed a field test of the NCS draft protocol prior to implementing the main study in the 105 locations. Our aim is to describe challenge, corrections, and formative research projects that provide empirical bases for establishing the final protocol for the NCS.

The original NCS sampling plan was to acquire a representative sample based on a random multi-stage cluster sample of locations and segments within locations with door-to-door recruitment of women of childbearing age to generate a sample of 1000 births per location. Home and clinic visits were performed to evaluate multiples procedures including questionnaires and environmental and biological specimens. Biospecimen repositories were established for future genomic and epigenomic analyses.

Recruitment: The rate of entry into the birth cohort was less than half the estimated rate. This identified major challenges for recruitment and retention that needed evaluation and new approaches. Three alternative recruitment schemes are currently in the field in 30 additional locations. In addition, formative research studies were designed to provide significant imput to protocol development. Environmental Exposure: Evaluation of environmental samples was tested for the analysis of collected environmental samples and inform analysis strategies. Pregnancy and Birth Visits: Measurements of stress during pregnancy were obtained and birth outcome were assessed. Genomics and Epigenomics: Blood samples from parent-child trios were accessed for feasibility studies of whole genome sequencing to establish background rates for nucleotide mutation and de novo copy number variations. Placenta samples were collected and evaluated for epigenomic alterations.

The Vanguard Centers of the NCS identified challenges to be addressed before the final NCS protocol is established. An expanded pilot phase was initiated, and extensive feasibility studies were initiated to provide an empirical basis for the next steps in sampling and recruitment strategies, assessment of biological and environmental samples that have been stored in repositories, and use of next-generation technology for genomic and epigenomic studies.

PI-025

Maternal C-Reactive Protein Is a Predictor of Neonatal Size at Birth: Pune Maternal Nutrition Study. D. S. Bhat[1], C. V. Joglekar[1], S. Rao[2], A. Kanade[2], H. G. Lubree[1], P. A Katre[3], K. J. Coyaji[1], C.H.D. Fall[4], C. S. Yajnik[1]. [1]*Kamalnayan Bajaj Diabetology Research Centre, King Edward Memorial Hospita Research Centre, Pune, India;* [2]*Biometry and Nutrition Unit, Agharkar Research Institute, Pune, India;* [3]*Persistent Systems Ltd, Pune, India;* [4]*MRC Epidemiology Resource Centre, University of Southampton, Southampton, United Kingdom.*

There is increasing interest in the role of maternal subclinical inflammation in fetal growth restriction. We studied the association between maternal high sensitive C-reactive protein (CRP) and offspring size at birth in Pune Maternal Nutrition Study (PMNS).

In PMNS we have information on prepregnant body size, adiposity, and socioeconomic status. Pregnancy measurements at 28 weeks gestation include anthropometry, physical activity, nutritional intake, circulating nutrients (vitamin B_{12}, folate, total homocysteine, vitamin C and vitamin D), biochemical measurements (CRP, oral glucose tolerance, hematology and lipids) and blood pressure (BP). Baby's anthropometry was measured within 72 hours of birth.

Results are available in 558 mother-offspring pairs. Before conception women were (median) 21y (19, 23), height 152 cm (149, 156), body mass index of 17.8 kg/m^2 (16.7, 19.1) and body fat 20.4% (17.8, 23.4). At 28 weeks, plasma CRP concentrations were 1.55 (0.68-3.44) mg/L and 28% had >3.0 mg/L. Gestation at delivery was 39.3 weeks (38.3, 40.3) and birth weight 2600 gm (2368, 2900). CRP was inversely related to age and parity and directly related to body fat percent ($p< 0.05$, all), but not with body mass index and socioeconomic status.

Pregnancy weight gain, physical activity, macronutrient (total calories, carbohydrate, proteins and fat) intake, circulating micronutrient (vitamin B_{12}, folate, vitamin C, vitamin D) concentrations, glucose tolerance, and lipids were not associated with CRP concentration. CRP was inversely associated with total homocysteine ($p=0.05$) and directly associated with systolic BP ($p=0.05$).

Mothers in highest quartile of CRP concentrations were 4.20 times (CI 1.64, 10.80) more likely to deliver preterm, and 1.68 times (CI 1.02, 2.76) more likely to deliver a low birth weight baby compared to those in lowest quartile. Association of maternal CRP and fetal growth restriction was independent of her age, adiposity, and parity.

Maternal total leucocyte count (TLC) not associated with preterm delivery and size at birth.

Maternal inflammation is a risk factor for early delivery and small size at birth in India.

PI-026

Withdrawn by Author

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PI-027

Weight Gain during Pregnancy and Offspring Birth Weight in the Western Region of São Paulo: The Butantã Cohort. Filumena Maria S. Gomes[1], Maria Helena Valente[1], Leide Irislayne M. Araujo[1], Luis Marcelo I. Cirino[2], Alexandra Brentani[1], Isac de Castro[1], Ana Maria U. Escobar[1], Sandra Josefina F.E. Grisi[1]. *[1]Pediatrics, Faculdade de Medicina da Universidade de São Paulo, SP, Brazil; [2]Surgery, Faculdade de Medicina da Universidade de São Paulo, SP, Brazil.*

The increasing prevalence of nutritional disorders in women during pregnancy and their role on pregnancy outcomes, such as fetal growth and birth weight, are related to the offspring health throughout life, and especially related to the presence of metabolic syndrome in adulthood. Excessive weight gain during pregnancy can have harmful consequences for both the mother and the fetus, such as mother's postpartum high weight, gestational diabetes, preeclampsia, dystocia, fetal macrosomia and increased need for surgical procedures. Insufficient fetus weight gain during pregnancy is related to low birth weight, higher morbidity and mortality in the first year of life and greater risk for developing diseases in adulthood, such as the metabolic syndrome.

Objective: To verify a correlation between offspring's birth weight and maternal weight gain during pregnancy.

A longitudinal and retrospective study of 1464 infants and their mothers followed in Butantã Cohort, from January 2007 to December 2009 was conducted. Butantã cohort is located in the western region of São Paulo City and is part of a research project of the Pediatrics Department. Maternal body weight gain was defined as: <8 kg=Insufficient; ≥ 8 to ≤ 12 kg=Appropriate; > 12 kg=Excessive. The cumulative frequency of weight was sequentially analyzed with Pearson's chi-square test for independent groups and expressed as proportions. Two tailed p values ≤0.05 were considered significant. Odds ratio for risk estimate was calculated.

14.94% (n=39) of women who had insufficient weight gain had newborn children weighing less than 2.500Kg, compared to 6,7 5 (n=81) who had appropriate or excessive weight gain showing a significant difference (p< 0.001). On the other hand, women who had insufficient weight gain had odds=2.43 (CI 1.62 to 3.66) of risk of having low birth children.

Insufficient maternal weight gain during pregnancy was significantly associated to lower birth weight of their offspring.

PI-028

Feasibility Studies in the National Choldrens Study: Genomics, Epigenomics, and Exposures. Kjersti Aagaard[1], Elaine Faustman[2], Debbie Nickerson[2], Ben Tycko[3], James Swanson[4]. *[1]OB/GYN, Baylor College of Medicine, TX, USA; [2]University of Washington, WA, USA; [3]Columbia University, NY, USA; [4]Pediatrics, UC Irvine, CA, USA.*

Specific genetic and epigenetic components were not specified in the original protocol for Vanguard Center phase of the National Childrens Study (NCS). Biological specimens were collected and repositories were established (e.g., for cord blood, placenta, etc.). Our aim is to describe feasibility studies intended to pfacilitate future genomic and epigenomic components of the NCS.

The Vanguard Center component is the NCS used a sampling plan intended to acquire a representative sample based on a random multi-stage cluster sample of locations (counties) and segments (neighborhoods) within locations with door-to-door recruitment of women of childbearing age from all households within the segments. Home and clinic visits were performed to evaluate procedure to the collection of environmental and biological specimens. Biospecimen repositories were established (e.g., for cord blood, placenta, etc.) for future genomic and epigenomic analyses.

Blood samples from parent-child trios were accessed for feasibility studies of whole genome sequencing to establish background rates for nucleotide mutation and de novo copy number variations. Placenta samples were collected and evaluated for epigenomic alterations using NexGen sequencing. Methylome analysis was undertaken with allele specific methylation. ChIP Seq with whole transcriptome shotgun sequencing (RNA Seq) was employed for functional characterization histone modifications with machine learning pipelines for the integration of these complex metadata. Real-time evaluation of environmental samples were designed to provide a larger array of options for the analysis of collected environmental samples and inform analysis strategies. Determination of environmental exposures using an "exposome" based approach is being evaluated.

The original Vanguard Center component of the NCS identified challenges that must be addressed before the final NCS protocol can be established. An expanded Vanguard Center pilot phase was initiated, and extensive feasibility studies were initiated to provide an empirical basis for the next steps in sampling and recruitment strategies, assessment of biological and environmental samples that have been stored in repositories, and use of next-generation technology for genomic and epigenomic studies.

PI-029

Arsenic Exposure and Folate Deficiency and Methyl Group Metabolism in Human Fibroblast Cell Lines. Etienne Gnimpiéba, Afif Abdel Nour, Latifa Abdennebi-Najar, Abalo Chango. *SNES-EGEAL, LaSalle Beauvais, France.*

Arsenic (*As*) is an environmental pollutant found in many water sources. The health consequences of chronic inorganic arsenic exposure include immune and endocrine disruption, reproductive dysfunction and adverse effects on fetal growth and development. *As* has been shown to induce DNA hypomethylation by continuous methyl groups demand in cells. We have evaluated *As* impact on methylation process pathways in human skin fibroblast cell line.

Cells were grown in media containing inorganic arsenic in folate depleted medium (FDM) or in experimental control medium (ECM) with optimal folic acid level. Methylation-Sensitive Arbitrarily Primed PCR method was used to evaluate the effect of *As* on DNA methylation. Folate receptors (FolR, RFC1) and DNA-methyltransferases (DNMT1, DNMT3a and DNMT3b) transcription levels were quantified.

FolR and *Rfc1* expresssion are both reduced in FDM. For, *As* decreased significantly *Rfc1* transcription in FDM compared to ECM. The fold-change for DNA-methyltransferase *Dnmt1*, expression was significantly increased in FDM compared to ECM. For *Dnm3a* and *Dnmt3b,* only *Dnmt3a* was highly expressed in ECM with *As*. No effect was observed in FDM. The study of cytokinesis-block micronuclei formation (CBMN) showed significant increase of micronucleus in cells grown in FDM and *As* (12.5 3.0%) than in ECM (8.1%+2.1%).

As has negative effect on the expression of *Rfc1* in FDM. Genomic DNA methylation profile and differential expression of DNMTs gene may suggest differences in maintaining methylation and *de novo* methyation. *A*s induced micronuclei formation that was exacerbate by folate deficiency which may be associated to chromosomal instability.

PI-030

Maternal Street Drug Use and Distress in Pregnancy, and Preschool Wheeze in Girls. Megan Alton[1], Suzanne Tough[3], Piushkumar Mandhane[2], Anita Kozyrskyj[2]. *[1]McGill University, Canada; [2]University of Alberta, Canada; [3]University of Calgary, Canada.*

Substance use during pregnancy is detrimental to child health. While asthma prevalence is high in offspring of substance-abusing mothers, nothing is known about the effect of substance use during pregnancy on the development of childhood atopic disease. We investigated the impact of maternal street drug use and distress during pregnancy on the development of wheeze and allergic disease in children.

Data were accessed from the Community Perinatal Care trial of 791 mother-child pairs in Calgary. This included information on maternal distress, alcohol, smoking, and street drug use during pregnancy, and child health outcomes collected via questionnaire at two time points during pregnancy and when children were three years old.

Using logistic regression, the association between maternal substance use and distress during pregnancy, and wheeze and allergy at age three was determined in boys and girls. Adjusting for alcohol and tobacco use during pregnancy, preterm birth, duration of exclusive breastfeeding, daycare attendance and maternal education level, maternal street drug use during pregnancy (OR:4.98, 95% Confidence Interval [CI]: 1.30-19.1), severe maternal distress during pregnancy (OR:5.81, 95% CI: 1.26-26.8) and prenatal vitamin use (OR:0.10, 95% CI:0.02-0.50) were associated with

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wheeze in girls. In boys, an independent association was found between severe distress during pregnancy (OR:3.61, 95% CI: 1.05-12.4) and allergies but none for maternal street drug use.
We found an association between maternal street drug use and wheeze in preschool girls that could not be explained by maternal distress, smoking or alcohol use during pregnancy.

PI-031

Substance Abuse and Low Birth Weight in the Western Region of São Paulo: The Butantã Cohort. Alexandra Brentani, Filumena Maria S. Gomes, Maria Helena Valente, Ana Maria U. Escobar, Isac de Castro, Sandra Josefina F.E. Grisi. *Pediatrics, Faculdade de Medicina da Universidade de São Paulo, SP, Brazil.*
Although many clinical manifestations of diseases appear only in adulthood, their origin may occur early in life. Considering this, the reduction of the low birth weight rate is a prioritie. This is due to the greater risk of morbidity and mortality that this condition brings, as well as the strong relationship established between underweight birth and consequent changes in the individual's gene expression, determining the later onset of chronic diseases, which are not only associated to physical exposure, but to social determinants during gestation, childhood and adulthood.
Objectives: determining if there is a correlation between parents's smoking habits and substance abuse and low birth weight.
Data from 1498 children born between 2007 and 2009, followed at Butantã Cohort, located in the western region of São Paulo was collected. The following information from patient's medical records were surveyed: gender, date of birth, place of residence, parental smoking habits and substance abuse, education level, gestational age, birth weight. Two groups of patients were formed: the study group, comprised of patients who had low birth weight (i.e., who weighed less than 2,500 g, and the control group, consisting of patients who had normal birth weight), (i.e., more than 2,500 g (WHO's definition)).Family habits and birth weight were correlated and statistically calculated. The cumulative frequency of the weight was sequentially analyzed with Pearson's chi-square test for independent groups and expressed in proportions. Two tailed p values ≤ 0.05 were considered significant.
16% (n=13) of low birth weight parents or responsible relatives declared drug addiction, while in the normal birth weight group only 5% (n=72) declared the same condition (p=0.0001). Concerning smoking habits, 56% (n=78) of low birth weight children's parents were smokers, while 42% (n=567) were smokers in the control group (p=0.0015). 30% (n=41) of low birth weight parents were alcoholic and 31% (n=417) in the other group representing no significant difference, the same happened to family wage levels and education level.
In this study we found a strong correlation between drug addiction or smoking in parents and low birth weight. The same pattern didn't happen in the case of alcohol addiction, family education and wage levels.

PI-032

Maternal Prenatal Smoking and Child Aggression: Exploring Intrauterine Effects in UK, Australian and Brazilian Cohorts. Marie-Jo A. Brion[1], Monique Robinson[2], Alicia Matijasevich[3], Colin Steer[4], Luciana Anselmi[3], Ana Maria B. Menezes[3], Craig Pennell[5], Lyle J. Palmer[6], Cesar G. Victora[7], George Davey Smith[1], Debbie A. Lawlor[1]. *[1]MRC Centre for Causal Analyses in Translational Epidemiology, School of Social and Community Medicine, University of Bristol, United Kingdom; [2]Telethon Institute for Child Health Research, The University of Western Australia, Australia; [3]Postgraduate Programme in Epidemiology, Federal University of Pelotas, Brazil; [4]Centre for Child and Adolescent Health, School of Social and Community Medicine, University of Bristol, United Kingdom; [5]School of Women's and Infants' Health, The University of Western Australia, Australia; [6]Ontario Institute for Cancer Research, University of Toronto, Canada.*
To determine if associations of maternal smoking and child aggression reflect causal intrauterine mechanisms.
We used UK (ALSPAC n=4604), Australian (Raine n= 1345) and Brazilian (Pelotas n=533) cohorts Intrauterine effects of maternal smoking on child aggression were explored using: 1) adjustments for measured confounders (socioeconomic position (SEP) and parental psychological factors); 2) maternal-paternal prenatal smoking comparisons, to assess unmeasured confounding; 3) cross-cohort comparisons of confounding structures; and 4) comparing maternal prenatal-postnatal smoking comparisons.
Associations of SEP with both maternal smoking and child aggression were weaker in Pelotas (Brazilian cohort) than either ALSPAC (UK) or Raine (Australia). Despite this, consistent associations of maternal prenatal smoking with greater child aggression were observed in all cohorts, independent of measured confounders. However, both the maternal-paternal comparisons and the prenatal-postnatal smoking comparisons indicated that associations of parental smoking with child aggression were not specific for maternal smoking in the prenatal period. Maternal smoking-offspring aggression associations were similar in magnitude to paternal smoking (at the time of their partners pregnancy)-offspring aggression associations and maternal postnatal smoking was associated with child aggression with a similar magnitude as, and independently of, pregnancy smoking.
Our evidence using multiple approaches to account for confounding suggests associations of maternal smoking with greater child aggression are not due to causal intrauterine effects, but are likely to reflect confounding by familial factors and, possibly, exposure to postnatal smoking.

PI-033

Excess of the Endocannabinoid Anandamide during Lactation, Induces Increased Levels of Its Receptor in Epididymal Fat, Overweight, and Insulin Resistance in Adult Mice. Carolina Aguirre, Valeska Castillo, Miguel Llanos. *Instituto de Nutrición y Tecnología de los Alimentos. Universidad de Chile, Santiago, Chile.*
Since type 1 cannabinoid receptors (CB_1R) have recently emerged as targets for modulating energy balance, overactivity of them during early life may result in undesirable metabolic consequences during adulthood. Central and peripheral activation of CB_1R by the endocannabinoid anandamide (AEA) increases appetite and energy storage, suggesting a role for overactive CB_1R in obesity and related metabolic consequences. The aim of this study was to evaluate long term effects of an excess of AEA during lactation on body weight and related metabolic parameters, epididymal fat (EF) accumulation and CB_1R and lipogenic factors expression in this tissue.
Twelve hours old, male mice pups were randomly distributed for maternal cross-fostering. During lactation, mice were orally treated with a solution of AEA (20 µg/g body weight) or vehicle. Food intake and body weight were recorded every 10 days. Adult animals were subjected to glucose tolerance and insulin sensitivity tests. Then, animals were sacrificed and EF extracted to evaluate mRNA expression of CB_1R and lipogenic enzymes by RT-PCR. Protein expression of CB_1R was performed by Western blot. Circulating levels of insulin, leptin, triglyceride and cholesterol were also evaluated.
AEA-treated mice during lactation showed a significant increase in accumulated food intake, body weight and EF during adulthood when compared to vehicle-treated animals ($p<0.05$; n=12; Mann-Whitney U test). Significant higher levels of circulating glucose, insulin, leptin, triglycerides and cholesterol were also observed ($p<0.05$). Moreover, a trend to glucose intolerance and a marked state of insulin resistance ($p<0.01$) were important findings in the AEA-treated group. Although CB_1R mRNA expression was not changed, CB_1R protein expression in AEA-group was 150% higher than control mice ($p<0.05$). Moreover fatty acid synthase mRNA expression was higher by 32% in AEA-treated mice ($p<0.05$).
These results show that overweight and associated metabolic disturbances such as insulin resistance and a higher lipid profile can be programmed in early stages of life by manipulating CB_1R with an elevated dose of its agonist AEA. This condition may be associated in part to the observed EF accumulation due to stimulation of lipogenic factors and the overexpression of CB_1R in adipose tissue. Supported by FONDECYT-CHILE 1100145

PI-034

Facial Responses to Primary Taste in Breast and Formula Feeding Infants. João G. Alves, Pedro Russo, Guilherme V. Alves. *Pediatrics, Instituto de Medicina Integral Prof Fernando Figueira (IMIP), Pernambuco, Brazil.*
To compare facial responses to primary four basic tastes (sweet, salt, bitter and sour) among breast and formula feeding infants.
Thirty healthy infants aged two months (15 exclusively breast feeding and 15 formula feeding) attending at Instituto de Medicina Integral Prof

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Fernando Figueira (IMIP) were studied. Each child was assessed with his mother in an isolated room with air conditioning (22 C). Caffeine (bitter), NaCl (salty), citric acid (sour) and sucrose (sweet) solutions were used to evaluate the primary taste. Each solution (0.2 cc) was applied on to the central portion of the dorsal surface of the infants' tongue. Distilled water was used as a rinse to minimize carry-over effects from one solution to the next. Their facial reactions were video recorded and analyzed using the Facial Action Coding System.
There were no differences in facial responses among breast and formula feeding. Negative responses were observed with bitter and sour solutions. Neutral and positive responses were observed with salt and sweet solutions.
Infants at age of two months, breast or formula feeding, do not show differences in facial responses to primary four basic tastes (salt, sweet, bitter and sour).

PI-035

Infant Feeding and Child Cognition and Behavior at Age 7 Years: Effects of Duration, Exclusivity, and Maternal Fish Intake. Mandy B. Belfort[1], Lauren B. Guthrie[2], Sheryl L. Rifas-Shiman[2], Elsie M. Taveras[2], Matthew W. Gillman[2], Emily Oken[2]. *[1]Newborn Medicine, Children's Hospital Boston, MA, USA; [2]Obesity Prevention Program, Dept of Population Medicine, Harvard Medical School/Harvard Pilgrim Health Care Institute, MA, USA.*
Our aims were: 1) to examine associations of duration of breastfeeding – any and exclusive -- with child cognition and behavior at age seven years; and 2) to examine the extent to which maternal fish intake during lactation modifies these associations.
We studied 1139 participants in Project Viva, a pre-birth cohort. When infants were six and 12 months old, we interviewed mothers about breastfeeding duration and exclusivity. At six months, we used a validated brief diet tool to assess mothers' fish intake since the infant's birth. At seven years, children completed the Kaufman Brief Intelligence Test (KBIT-II), Wide Range Assessment of Visual Motor Abilities (WRAVMA) drawing subscale, and Wide Range Assessment of Memory and Learning (WRAML), and mothers completed the Behavior Rating Inventory of Executive Function (BRIEF) and Strengths and Difficulties Questionnaire (SDQ), a measure of child behavior. In multivariable linear regression, we adjusted for sociodemographic characteristics, maternal intelligence, and the Home Observation Measurement of the Environment score.
Mean ±standard deviation (SD) duration of any breastfeeding was 6.45 ±4.6 months and of exclusive breastfeeding was 2.84 ±2.5 months. Mean ±SD KBIT-II verbal score was 112.5 ±14.7; KBIT-II nonverbal 106.5 ±16.8; WRAVMA 92.2 ±16.8, WRAML 16.9 ±4.4; BRIEF 48.5 ±9.1; and SDQ 6.4 ±4.7. Greater duration of any breastfeeding was associated with higher verbal KBIT-II scores at age seven (0.4 points/month breastfed, 95% CI 0.2, 0.6), but not with the other cognitive or behavioral measures. Greater duration of exclusive breastfeeding was also associated with higher verbal KBIT-II scores (0.8 points/month, 95% CI 0.4, 1.1), and with higher WRAML scores (0.2 points/month, 95% CI 0.1, 0.3). Associations were not stronger for children of mothers with fish intake >2 servings/week.
Greater duration and exclusivity of breastfeeding may improve verbal intelligence, memory, and learning at age seven years. Maternal fish intake >2 servings/week during lactation did not strengthen these associations.

PI-036

Duration of Breastfeeding and Child Neurodevelopment: Results of the EDEN Mother-Child Cohort. Jonathan Y. Bernard[1,2], Maria de Agostini[1,2], Anne Forhan[1,2], Toni Alfaiate[1,2], Mercedes Bonet[3,4], Laetitia Marchand[3,4], Béatrice Blondel[3,4], Valérie Champion[3,4], Monique Kaminski[3,4], Blandine de Lauzon-Guillain[1,2], Marie Aline Charles[1,2], Barbara Heude[1,2]. *[1]UMRS 1018 Epidemiology of Diabetes, Obesity and Chronic Kidney Disease Over the Lifecourse, INSERM, Center for Research in Epidemiology and Population Health, Villejuif, France; [2]Faculty of Medicine, University Paris-Sud, Kremlin-Bicetre, France; [3]INSERM U953 Epidemiological Research Unit on Perinatal Health and Women's and Children's Health, France; [4]UPMC University Paris 6, France.*
Studies on the role of breastfeeding (BF) on child neurodevelopment are controversial. Most of them investigated children of 5y and over. Associations may be easier to demonstrate in early childhood, closer to the exposure period, taking into account BF duration to investigate a possible dose-effect relationship. Our objective was to explore the associations of BF duration with neurodevelopment at two and 3y, after controlling for a wide range of confounders.
In children born at term of a French mother-child cohort, we investigated language ability at 2y with the MacArthur-Bates Communicative Development Inventories (CDI, n=1350) and global development at 3y with the Ages and Stages Questionnaire (ASQ, n=1081). Durations of exclusive BF and any (exclusive or mixed) BF were recorded in months and investigated separately in association with each developmental assessment. Multiple linear regressions were used to adjust for centre, and covariates characterizing the child (age, sex, gestational age), the mother (age, education, bmi, tobacco and alcohol consumption in pregnancy) and the social environment (income, siblings, caregivers, story reading frequency, preschool attendance).
On average, duration of exclusive and any BF were of respectively 2.6 and 4.6 months. Scores were significantly higher in exclusively breastfed children than in never breastfed (+5.3 points, $p<0.01$ and +7.8, $p<0.001$ respectively for CDI and ASQ). In breastfed children, duration of exclusive BF was significantly associated with increased CDI ($\beta=0.73\pm0.34$ points/month, $p<0.05$) and ASQ scores ($\beta=0.98\pm0.37$, $p<0.01$). For any BF duration, the regression coefficients were respectively 0.63 (±0.25, $p<0.05$) and 0.83 (±0.27, $p<0.01$). The associations did not differ according to gender.
After controlling, there were positive linear associations between duration of BF and both language at 2y and global development at 3y, suggesting a dose-effect relationship.

PI-037

Dietary Choline Intake during Lactation Impacts the Growth of Offspring. Neele Dellschaft, Rene Jacobs, Susan Goruk, Nicole Coursen, Jonathan Curtis, Catherine J. Field. *Agricultural, Food and Nutritional Sciences, University of Alberta, AB, Canada.*
Although it has been established that choline is an important nutrient during pregnancy, the essentiality of choline (or the dietary forms of choline) in the maternal diet during the suckling period has not been established and is the purpose of the current study.
Sprague-Dawley dams (n=4/group) were fed a high-fat, isocaloric diet from delivery until the end of the suckling period (21d), containing phosphatidylcholine (PC), choline (C) or neither (D). Similar to our earlier studies, D mothers gain less weight during lactation. At 21d, stomach contents were collected (n=2/dam) and analysed by LC-MS for choline metabolites. Two female pups per dam were weaned to a high-fat diet (20% w/w) containing 1g/kg choline as free choline and body weight and food intake were measured regularly until 11 wks of age.
There was a lower concentration of free choline in D (0.3 ± 0.1mg/g) compared to C (6.1 ± 1.0) and a higher PC concentration in the PC group (66 ± 13mg/g) compared to D (35 ± 13) and C (24 ± 3), $p<0.05$, suggesting that endogenous synthesis of PC is reduced in D mothers. Length and body weight for D pups was significantly lower than that of PC and C at four wks (weight: PC, 90 ± 1; C, 89 ± 2; D, 66 ± 2g; $p<0.05$) and five wks of age (PC, 137 ± 5; C, 135 ± 4; D, 116 ± 3g; $p<0.05$) but did not differ at 7 wks. This catch up growth was likely due to a higher food intake adjusted for body weight (wk 4, PC, 140 ± 2; C, 120 ± 5; D, 166 ± 13 mg diet/g body weight*d; for PC vs. C, C vs. D $p<0.01$) by the D pups. At 9 wks of age, an intraperitoneal glucose tolerance test (IPGTT) was performed but insulin and glucose response (area under the curve) did not differ significantly among groups.
Our findings suggest that a source of choline is required in the maternal diet during suckling to provide choline for maternal milk and to induce normal growth in the offspring. The effects of maternal choline deficiency on pup body weight remained for several weeks, despite feeding a choline sufficient diet. Rapid catch-up growth occurred by 7-8 wks of age but glucose tolerance at 9 wks was not significantly affected. Currently, studies are underway in these animals to determine the effects of early choline deprivation on immune function and appetite regulatory pathways in the hypothalamus later in life.
(Funded by ALMA, Alberta Egg Producers and NSERC.)

PI-038

The Challenges of Large Scale Epigenetic Methylation Data: Statistical Explorations into Acceptability of Replicates. Sheila J. Barton[1], Hazel M. Inskip[1], Abigail Lapham[2], Emma Garratt[2], Graham C. Burdge[2], Karen A. Lillycrop[2,3], Mark A. Hanson[2,4], Cyrus Cooper[1], Keith M. Godfrey[1,4]. *[1]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom; [2]Human Development and Health Academic Unit, United Kingdom; [3]School of Biological Sciences, United Kingdom; [4]NIHR Nutrition, Diet & Lifestyle Biomedical Research Unit, University of Southampton, United Kingdom.*

Whole genome promoter methylation arrays can generate >500,000 data points for each sample analysed. It is common practice to submit replicate samples to check measurement accuracy, however due to the large number of data points it is not obvious what constitutes an acceptable replicate. We used statistical techniques to assess the acceptability of replicates produced by such a process.

28 perinatal DNA samples were submitted to an Agilent Comparative Genomic Hybridization array; of these two had been divided into three and submitted with different id numbers. The distributions of values obtained across each array were examined and standard deviation (SD) between and within replicates calculated using variance components. Principal components analysis was used to produce component plots showing clustering of replicate samples. Hierarchical clustering methods demonstrated similarities or otherwise between replicate samples. Measures of agreement (Kappa) values were calculated for pairs of samples.

Distributions of replicate samples were not always similar. The SD between subjects was greater than the SD within subjects for 54% of a random sample of probes, suggesting that biological variability is greater than measurement variability for these probes. Component plots indicated that one of the replicates did not cluster with the others. Hierarchical clustering confirmed the results from the plots. Kappa values (0.02 to 0.22) sometimes indicated more agreement between different samples than between replicates.

Submitting replicate samples for methylation arrays provides a check on the validity of data obtained. However it is not trivial to determine a cut-off level of acceptability of similarity of replicates. Statistical techniques can indicate replicate samples that are clearly different from each other; but it is more difficult to specify how similar replicate samples should be for acceptability. The findings emphasize the importance of using sequencing techniques to confirm or refute findings from methylation arrays.

PI-039

Maternal Expression of n-3 Desaturase Provides Protection from Fetoplacental Lipotoxicity in a Mouse Model of Obese Pregnancy. Margaret Heerwagen[1], Becky De la houssaye[1], Rachel Janssen[1], Bryan Bergman[2], Jacob Friedman[1]. *[1]Pediatrics/Neonatology, University of Colorado, Anschutz Medical Campus, CO, USA; [2]Edocrinology, University of Colorado, Anschutz Medical Campus, CO, USA.*

Maternal obesity negatively impacts fetal development and increases risk for later metabolic disease; this may be due to excess fetal lipid exposure and resultant lipotoxicity. To determine if an increased maternal omega-3/omega-6 ratio can prevent these adverse maternal-fetal outcomes we utilized the Fat-1 mouse.

The Fat-1 mouse expresses a novel c.elegans-derived n-3 desaturase enzyme under the beta actin promoter, thus increasing tissue omega-3 levels without increasing omega-3 intake. Wild type (WT) and Fat-1 female litter mates were fed a 45% high-fat diet (HFD) or a 10% low-fat control diet for 8 wk prior to mating. Pregnant females were maintained on their respective diets through gestation, and fetuses examined on E18.5 (n=8 mothers per experimental group). Only WT pups were used in analysis.

Fetuses from WT HFD mothers demonstrated a significant ($p<0.03$) 100% and 80% increase in TG deposition in the placenta and fetal liver, respectively, which was prevented in fetuses from Fat-1 HFD mothers despite maternal obesity. Further, placental and fetal liver omega-3/omega-6 ratios were increased in WT fetuses from Fat-1 HFD mothers, due to a significant doubling ($p<0.01$) of DHA levels. Additionally, pro-inflammatory cytokine levels in maternal and fetal serum were elevated in WT HFD mothers and their offspring, and this was associated with increased placental and fetal liver cytokine gene expression ($p<0.03$), which was normalized in the Fat-1 HFD group. Interestingly, increased expression of lipid oxidative genes was only observed in the placenta of WT HFD mothers ($p<0.03$), but in the fetal livers of Fat-1 HFD mothers ($p<0.05$). Such differences suggest HFD exposure triggers metabolic programming in the placenta, but may not provide adequate protection in the fetus.

Maternal HFD triggers fetal hepatic lipotoxicity pathways in-utero. The n-3 desaturase enzyme provides a powerful protective effect on maternal-fetal inflammation and excess placental and fetal lipid accumulation despite HFD-induced obesity. This approach may hold promise for reducing maternal obesity-associated fetal lipotoxicity and its negative effects on fetal metabolic health.

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PI-040

Association of *FUT2* and *TCN2* Gene Polymorphisms with Maternal B_{12} Levels and Offspring Birth Weight and Insulin Resistance: Data from Two Indian Birth Cohorts. C. V. Joglekar[1], C. S. Yajnik[1], C. Spurgeon[2], C. Fall[3], G. Krishnaveni[4], S. Veena[4], K. Mani[2], M.V.K. Kumar[2], G. R. Chandak[2]. *[1]Diabetes Unit, King Edward Memorial Hospital & Research Centre, Pune, Maharashtra, India; [2]Centre for Cellular & Molecular Biology, Hyderabad, Andhra Pradesh, India; [3]MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, United Kingdom; [4]Epidemiology Research Unit, Holdsworth Memorial Hospital, Mysore, India.*

Genome-wide association studies have shown an association between *FUT2* and *TCN2* polymorphisms and circulating vitamin B12 concentrations. We investigated the associations of these polymorphisms with parameters of 1-C metabolism in mothers and their influence on offspring birth weight, adiposity and insulin resistance in two Indian birth cohorts.

We genotyped 1196 mothers in two prospective studies of maternal nutrition and fetal growth (Pune Maternal Nutrition Study (PMNS, n=671) and Parthenon Study, (n=525)) for polymorphisms rs492602 (c.204A→G), rs601338 (c.461G→A), and rs602662 (c.772G→A) in *FUT2* gene and rs1801198 (c.776C→G) in *TCN2* gene. Circulating vitamin B12, folate and homocysteine concentrations in mothers at 28 weeks gestation and the birth weight of the children were available in the database. Children were investigated at six years of age for glucose-insulin metabolism.

Fifty eight percent of mothers had low vitamin B_{12} status (<150 pmol/L), 2% had low folate status and 19% percent were hyperhomocysteinemic (>10μmol/L). Rare allele frequencies for different polymorphisms varied from 10 to 15%. All three *FUT2* polymorphisms were in strong linkage disequilibrium ($D'=0.99$; $r^2=0.93$) and risk alleles at all three were associated with higher maternal vitamin B_{12} concentrations *TCN2* polymorphism was not associated. Interestingly all *FUT2* variants were inversely associated with maternal folate concentrations ($P<0.001$) but none were associated with plasma total homocysteine concentrations. *FUT2* polymorphisms were not associated with offspring birth weight but the risk allele in *TCN2* gene predicted lower birth weight (per allele there was 43gm , 0.07SD unit decrease in birthweight). Risk allele at rs601338 in *FUT2* in the mother predicted lower insulin resistance in the offspring at 6y.

FUT2 gene polymorphisms predict vitamin B12 concentrations in Indians. The association between maternal genotype and offspring birth weight and insulin resistance suggests a causal programming influence of maternal vitamin B_{12} nutrition on fetal programming.

PI-041

Telomere Shortening Correlates with Programming of the Metabolic Syndrome Phenotype. Richard Maganga[1], Lauren Chun[2], Craig E. Pennell[1], Stephen J. Lye[2,3]. *[1]School of Women's and Infants' Health, The University of Western Australia, Western Australia, Australia; [2]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Canada; [3]Obstetrics & Gynaecology, The University of Toronto, Canada.*

Impaired fetal nutrition has long-term adverse effects on adult health by programming the development of adult diseases such as the metabolic syndrome, in addition to compromising life span in animal models. Telomeric DNA loss is associated with stress and age-related disease, and telomere length is used as a predictor of biological aging. We have previously characterized the impact of maternal dietary restriction during pregnancy on fetal phenotype in two phylogenetically distinct mouse strains, *C57BL/6J* (B6) and *A/J*. Calorie-restricted B6 mice were more susceptible to developing an adverse metabolic phenotype than *A/J* mice.

We hypothesize that telomere shortening may represent a mechanism by which early life events are associated with developmental programming of the metabolic syndrome.
Pregnant B6 and A/J mice were subjected to either 30% dietary restriction from day 6.5 to 17.5 of gestation (B6R, AJR) or ad libitum food access (B6C, AJC). Mouse kidneys and livers were collected at day18.5 of pregnancy (near term) or at six months of age for genomic DNA isolation. Telomere length was analysed by quantitative real-time PCR normalized against a single copy gene.
At day 18.5 of pregnancy, telomere length in B6R was shortened by up to 50% in both kidney and liver compared to the B6C, with larger effects in males than females. Conversely, no significant telomere shortening was seen in AJ fetuses exposed to maternal dietary restriction. Telomere shortening was apparent at six months of age in both strains with B6R having significantly shorter telomere length than AJR, possibly reflecting the greater susceptibility of the B6 strain to adverse consequences of maternal dietary restriction.
In mice, reduced telomere length correlates with the development of the metabolic phenotype in adulthood in response to maternal undernutrition during pregnancy. The shorter telomeres in the B6 strain at birth suggest that telomere length may contribute to susceptibility to developmental programming and may be a useful marker for determining individuals at risk for the metabolic syndrome phenotype.

PI-042

The Role of Genomic Imprinting in DOHaD. Karin B. Michels. *Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.*
The intrauterine origin of several chronic diseases including cardiovascular disease, type 2 diabetes mellitus, neuropsychiatric disorders, and cancer is well established. Epigenetic aberrations, specifically changes in genomic imprinting, have been implicated to provide a mechanistic explanation for these associations. Maternal starvation has been linked to altered DNA methylation of the insulin-like growth factor 2 (IGF2) gene in the offsprings' blood in adulthood.
We used biospecimens from a birth cohort at Brigham and Women's Hospital, Harvard Medical School, in Boston to examine the DNA methylation of imprinted genes in the cord blood of newborn infants. We also examined DNA methylation of imprinted genes in the tissue and cord blood of women with and without invasive breast cancer. Bisulfite pyrosequencing was used to assess DNA methylation at the imprinting control region of IGF2 (IGF2DMR0).
Among several hundred samples of cord blood with considerable variation in birthweight and maternal weight parameters, no hypo- or hypermethylation of the IGF2DMR0 was found. Conversely, hypomethylation of the IGF2DMR0 was frequently observed in the mammary tissue of women with invasive breast cancer. IGF2DMR0 was not hypomethylated in the blood of women with invasive breast cancer.
Loss of imprinting of IGF2 found in cancer tissue is likely acquired during the lifecourse as a results of environmental exposures. Loss of imprining of IGF2 in utero is an extremely rare phenomenon and associated with the severe phenotypes of rare childhood disorders. Intrauterine conditions are unlikely to affect DNA methylation of IGF2 without resulting in a severe childhood phenotype.

PI-043

Maternal Weight, Birthweight, and Global DNA Methylation at Birth. Karin B. Michels, Holly R. Harris, Ludovic Barault. *Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.*
Low birthweight, premature birth, intrauterine growth retardation, and maternal malnutrition and have been related to an increased risk of cardiovascular disease, type 2 diabetes mellitus, obesity, and neuropsychiatric disorders later in life. Conversely, high birthweight has been linked to future risk of cancer.
We used data and biospecimens from an epigenetic birth cohort at Brigham and Women's Hospital, Harvad Medical School, in Boston, to explore the association between trajectories of fetal and maternal growth and LINE-1 methylation in 319 mother-child dyads.
Newborns with low or high birthweight had significantly lower LINE-1 methylation levels in their cord blood compared to normal weight infants after adjusting for gestational age, sex of the child, ethnicity, maternal age at delivery, and maternal smoking during pregnancy (p=0.007 and p=0.036, respectively), but the magnitude of the difference was small. Infants born prematurely also had lower LINE-1 methylation levels in cord blood compared to term infants, and this difference, though small, was statistically significant (p=0.004). We did not find important associations between maternal growth trajectories and global methylation of the cord blood or fetal placental tissue.
We found significant differences in cord blood LINE-1 methylation among newborns with low and high birthweight as well as among prematurely born infants. Future studies may elucidate whether chromosomal instabilities or other functional consequences of these changes contribute to the increased risk of chronic diseases among individuals with these characteristics.

PI-044

Undiagnosed Diabetes and Prediabetes among Adults in Urban Dhaka and Rural Matlab, Bangladesh: A Hidden Public Health Burden. Dewan S. Alam[1], Shamim H. Talukder[2], Mohammad Yunus[1], Tracey L. Koehlmoos[1], Alejandro Cravioto[1], Louis W. Niessen[1]. *[1]CCCDB, ICDDR,B, Dhaka, Bangladesh; [2]Eminence, Dhaka, Bangladesh.*
To identify undiagnosed diabetes and prediabetes cases in adults 20 years and above living in urban and rural settings.
The study was conducted in Mirpur, an urban middle class area in Dhaka city and in rural Matlab, Bangladesh. Participants were adults males and females 20 years and older. Data were collected using a structured questionnaire. Glucose metabolic status was measured at fasting and two hours after the administration of 75g of oral glucose using a HemoCueTM glucometer. We used standard cut-off values to identify diabetic and prediabetic individuals.
In total, 1243 individuals, 517 from urban Mirpur and 726 from rural Matlab participated in the study. Mean age of the participants was 41 years and average BMI of 23 kg/m2 with the urban participants being heavier than rural ones. Diabetes prevalece was significantly higher among urban than rural parcicipants (12.0% vs 2.7%, $p<0.001$) but no difference was seen between males and females in both areas. Prediabetes rates were also higher in the urban population (19.1% vs 12.7%, $p<0.05$). In the rural sample females had higher prediabetes rate than males. Highest prevalence of both diabetes and prediabetes was observed in individuals who had combined high BMI and high waist circumference.
Diabetes and prediabetes are alarmingly high in urban middle class population. This unexpected rate of diabetes and prediabetes in people who otherwise consider themselves as normal and healthy requires immediate attention and should be considered in any diabetes primary prevention programme in Bangladesh. Weight reduction intervention in overweight individuals needs to be addressed in people with abdominal obesity.

PI-045

Chronic Placental Insufficiency in Pregnant Sheep Produces IUGR and Reduced Leucine Flux into Fetal Tissues. Laura D. Brown, Paul J. Rozance, Stephanie R. Thorn, Jacob E. Friedman, William W. Hay. *Pediatrics, University of Colorado Denver, CO, USA.*
Background Placental insufficiency results in decreased leucine flux into the fetal circulation, decreased fetal plasma insulin concentrations, and poor fetal growth. Studies that reintroduce amino acids (AA) and/or insulin to the IUGR fetus might reveal tissue-specific resistance to the activation of growth pathways.
Objective
To determine whether chronic placental insufficiency results in decreased insulin and AA-stimulated fetal protein accretion and limited activation of the mTOR signal transduction pathway in fetal skeletal muscle.
Methods IUGR was produced using a sheep model of placental insufficiency and reduced fetal nutrient supply and compared to normal controls (C). On day 132, IUGR and C fetuses received one of three treatments: 1) 3 hr infusions of mixed AA to double fetal [AA] plus somatostatin to suppress AA-stimulated insulin secretion (IUGR, n=5 and C, n=4), 2) insulin with euglycemia and euaminoacidemia (IUGR, n=6 and C, n=5), or 3) saline alone. Baseline, AA-stimulated, and insulin-stimulated rates of net fetal substrate uptake and fetal leucine metabolism were measured followed by a skeletal muscle biopsy to quantify mTOR signaling.

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Results IUGR fetuses had lower weights (40%), decreased arterial glucose (40%), insulin (55%), and O2 contents (40%), and decreased rates of umbilical blood flow per kg (20%), net fetal O2 uptake (10%), leucine uptake (25%), leucine disposal (20%), and leucine flux into fetal tissues (20%) compared to C fetuses ($P<0.05$). AA-induced leucine flux into fetal tissues increased by 35% in C fetuses only ($P<0.01$). AA increased leucine oxidation in both groups, but to a greater extent in C (100% vs. 25%, $P<0.05$). Insulin and AA effects on protein synthesis, accretion, and signaling through Akt-mTOR-p70S6k pathway did not differ between C and IUGR fetuses.

Conclusions IUGR fetuses have lower rates of leucine flux into fetal tissues at baseline and in response to an acute AA infusion, unique evidence of tissue resistance to reintroduction of AA when concurrent insulin secretion is suppressed. Leucine that did enter fetal tissues was oxidized more in C than IUGR fetuses. Stimulation of mTOR signaling in IUGR muscle is intact, further supporting that defective tissue AA uptake limits AA metabolism in chronically nutrient-deprived IUGR fetuses.

PI-046

Maternal Gestational Dietary Fat Effects on Hepatic GLUT2 and Glucokinase Expression and on Plasma Lipid Profiles in Neonatal Wistar Rat Offspring. Marlon E. Cerf, Keith Williams, Christo J. Muller, Johan Louw. *Diabetes Discovery Platform, South African Medical Research Council, Western Cape, South Africa.*

To investigate the effects of maternal diets, varying in fat content, on circulating glucose, insulin, glucagon and lipid profiles and on the expression of the hepatic glucose sensing factors, glucose transporter 2 (GLUT2) and glucokinase (GK) in neonatal offspring.

Dams were maintained on diets of 10% (control), 20% (20F), 30% (30F) and 40% (40F) fat as energy throughout gestation. Maternal daily food intakes and weekly body weights were measured. Circulating glucose, insulin and glucagon concentrations were determined in the dams and their offspring. In neonatal offspring, total plasma triglyceride, total and individual plasma fatty acid concentrations, hepatic GLUT2 and GK mRNA (quantitative PCR) and protein (immunostaining and image analysis) expression were determined.

In mothers, overall food intake of the 20F and 40F mothers was reduced compared to both the control and 30F mothers with no significant changes in circulating glucose, insulin or glucagon concentrations. However 40F mothers displayed a 2.6 fold increase in circulating insulin concentrations. The 20F neonates displayed elevated blood glucose concentrations. The 40F neonates displayed a 1.9 fold increase in circulating insulin and 0.58 fold reduction in glucagon concentrations. Total triglyceride and total free fatty acid concentrations were not altered after dietary intervention in neonates. No significant changes in either hepatic GLUT2 and GK mRNA expression or immunoreactivity were demonstrated when comparing the 20F, 30F and 40F neonates to control neonates. However GLUT2 immunoreactivity was increased in the 40F neonates relative to the 20F and 30F neonates.

Nutritional programming with different dietary fat proportions has a minimal influence neonatal hormonal and lipid profiles and on the hepatic expression of the glucose sensing factors, GLUT2 and GK. Elevated glucose concentrations in 20F neonates and the approximate doubling of circulating insulin concentrations in the 40F neonates reflect an insulin resistant state in these offspring demonstrating some systemic effects induced by nutritional programming.

PI-047

Association Analysis of *ADCY5* and *CCNL1* Gene Polymorphisms with Birth Weight and Insulin Resistance in Two Large Indian Birth Cohorts. S. Priyadarshini[1], Smita R. Kulkarni[2], G. D. Vinay[1], Radha K. Mani[1], V. G. Krishnaveni[3], R. S. Veena[3], Charu V. Joglekar[2], Caroline H.D. Fall[4], Chittaranjan S. Yajnik[2], Giriraj R. Chandak[1]. *[1]Centre for Cellular and Molecular Biology, India; [2]Diabetes Unit, KEM Hospital and Research Centre, India; [3]Epidemiology Research Unit, Holdsworth Memorial Hospital, India; [4]MRC Lifecourse Epidemiology Unit, University of Southampton, India.*

Indians are born with low birth weight, which is an established risk factor for future development of type 2 diabetes (T2D). Recent genome-wide association studies have identified two loci, *ADCY5* and *CCNL1* that influence birth weight. We investigated whether variants in these loci have any impact on birth weight, adiposity and future risk of T2D in two Indian birth cohorts.

A total of 1196 children from two birth cohorts (Pune Maternal Nutrition Study (n=671) and Parthenon Study (n=525) were genotyped for rs9883204 in *ADCY5* and rs900400, near *CCNL1* genes. We investigated the association of these polymorphisms with birth weight and other anthropometric parameters and for glucose-insulin metabolism at six years of age.

The average birth weight was 2.74 (+/-0.42) kg; 31.4% of children were low birth weight (LBW; ≤2.5 kg) and 11.2% were small for gestational age (SGA; <10th centile). The risk allele frequency for the variants was comparable to those reported in the Europeans. We did not observe any significant association of variants in *ADCY5* (p=0.42) or *CCNL1* (p=0.12) or both together (p>0.05) with birth weight (gestation, gender and study specific) even after adjusting for parity, maternal BMI and socioeconomic status (SES). Similar observations were made on analysis based on their LBW and SGA status. Interestingly, the weight lowering allele of *CCNL1* predicted lower ponderal index (PI; beta-0.076, p=0.012) and subscapular thickness (beta=0.055, p=0.045) but *ADCY5* variant had no influence (even on adjusting for parity, SES, maternal BMI). Addition of both SNPs in a model still showed that the variant near *CCNL1* influences PI, subscapular and triceps thickness. The risk allele at *ADCY5* variant, which is also a T2D risk locus, had no influence on insulin resistance at six years of age.

The birth weight-lowering genetic variants near *CCNL1* and *ADCY5* had no influence on the birth weight in Indians but predicted lower fat mass. This may suggest a predominant role of environmental factors including maternal micronutrient status or other genetic factors.

PI-048

Pre-Pubertal Baboon Offspring of Mothers Exposed to Moderate Maternal Nutrient Restriction (MNR) in Pregnancy and Lactation Show Peripheral Insulin Resistance. Jaehyek Choi[1], Thomas J. McDonald[1], Anthony G. Comuzzie[2], Vicki Mattern[2], Susan L. Jenkins[1], Cun Li[1], Peter W. Nathanielsz[1]. *[1]Center for Pregnancy and Newborn Research, Dept. OB/GYN, The University of Texas Health Science Center San Antonio, TX, USA; [2]Dept. Genetics, Texas Biomedical Research Institute, TX, USA.*

Moderate MNR (30% reduction in intake) impairs fetal pancreatic (AJOG 2008: 215). Prior to puberty offspring show a decreased IVGTT insulin (SGI 2011: F-150). To determine whether insulin resistance is also present we conducted a hyperinsulinemic euglycemic insulin clamp (HIEC). We hypothesized that moderate MNR would result in developmental programming of offspring peripheral insulin sensitivity predisposing to emergence of a pre-diabetic state prior to puberty.

Non pregnant female baboons (11.5 ± 0.5 y) of similar morphometric phenotype were randomly fed control *ad lib* diet (CTR, n= 12) or MNR (n=6; 70% CTR diet) through pregnancy and lactation. After weaning OFF ate normal chow. At 3.5 ± 0.18 y, offspring femoral artery and vein were catheterized under general anesthesia and baboons, placed on a tether system and recovered for at least a week before conducting a HIEC while conscious (insulin infused at 60 $mU.m^{-2}$•min^{-1} to raise plasma insulin by approx 100 $iU.ml^{-1}$.) After the start of the insulin infusion, a 20% glucose infusion was begun and plasma glucose measured every 5 min to adjust the glucose infusion rate to maintain plasma glucose of 90 $mg.dl^{-1}$and plasma insulin and c-peptide measured every 15 min. Two final samples were taken at 115 and 120 min to ensure equilibrium. Group comparisons by Student's t-test; Data M±SEM, α 0.05.

Blood glucose, insulin and C-peptide were not different in the two groups during the clamp. Glucose disposal rate was reduced ($p < 0.05$) in MNR offspring ($mg.kg^{-1}$.min. (CTR; 29.1±1.58 $mg.kg^{-1}$.min; n=12 , MNR; 22.1±3.07 $mg.kg^{-1}$.min; n=6,).

Glucose disposal rate was decreased in baboon offspring exposed to moderate MNR in development. This early emergence of insulin resistance occurred even without the second hit of a western diet. Supported by NIDDK1R21DK085420-01 and R24RR21367.

PI-049

Evidence of Hepatic Insulin Resistance in Fetal C57BL/6J Mice Subjected to Maternal Dietary Restriction. Lauren A. Chun[1,2], Brian S. Knight[1,2], Craig E. Pennell[3], Stephen J. Lye[1,2]. [1]*Samuel Lunenfeld Research Institute, Mount Sinai Hospital, ON, Canada;* [2]*Obs/Gyn and Physiology, University of Toronto, ON, Canada;* [3]*School of Women's and Infants' Health, University of Western Australia, Perth, Australia.*

Reduced maternal dietary intake during gestation is associated with the development of the metabolic syndrome in adults (characterized by obesity, atherogenic dyslipidemia, hypertension, and insulin resistance). Our laboratory has established a mouse model of developmental programming where maternal caloric restriction of C57BL/6J (B6) mice produces fetuses that are growth-restricted and adult male offspring which develop the metabolic syndrome. The mechanism for this association has not yet been fully charcterized. We aim to investigate the impact of maternal undernutrition on the fetal hepatic gluconeogenic pathway and insulin sensitivity.

Pregnant B6 mice were fed either ad libitum as controls (C) or a 30% calorie-reduced diet (R) from gestational day (d) 6.5 to 17.5. In study 1 (glucose study), fetal (d18.5) trunk blood glucose was measured (N = 6 to 7/group) together with hepatic expression of glucose 6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase-1 (PCK1), pyruvate carboxylase (PC) (N = 6/group). RNA and protein expression were evaluated using Real-time PCR and immunoblotting, respectively. In study 2 (insulin study), an intraperitoneal injection of 30 μCi of [U-14C]D-glucose was administered on d18.5 (N = 5 to 6/group). After six hours, mice were euthanized and glycogen was isolated from fetal liver tissue to detect the incorporation of [U-14C]D-glucose.

In study 1, offspring of dietary restricted mothers synthesized less glycogen (-72%, $p<0.001$) compared to controls despite having elevated resting glucose levels (+33%, $p<0.05$), elevated gluconeogenic enzyme RNA expression (G6Pase: +1044%, $p<0.001$; PCK1: +818%, $p<0.001$; PC: +53%, $p<0.05$) and increased protein expression (PCK1: + 247%, $p<0.001$). In study 2, offspring from dietary restricted mothers synthesized less glycogen compared to controls (+72%, $p<0.0001$).

Increased expression of hepatic gluconeogenic enzymes reveals fetal adaptation to poor maternal nutrient provision. The reduction in fetal glycogen synthesis by restricted offspring despite elevated resting blood glucose suggests that hepatic insulin resistance begins to develop in utero.

PI-050

Stability of Lipoprotein Lipase Promoter Methylation Levels in Peripheral Blood Samples. Paula M. Costello[1], Marjolein V.E. Veenendaal[2], Samuel P. Hoile[1], Karen A. Lillycrop[1], Graham C. Burdge[1], Susanne R. de Rooij[2], Rebecca C. Painter[3], Sheila J. Barton[4], Joris A. van der Post[3], Patrick M. Bossuyt[2], Peter D. Gluckman[5], Mark A. Hanson[1], Tessa J. Roseboom[2]. [1]*Human Development and Health, University of Southampton, United Kingdom;* [2]*Department of Clinical Epidemiology and Biostatistics, University of Amsterdam, Netherlands;* [3]*Department of Obstetrics and Gynaecology, University of Amsterdam, Netherlands;* [4]*MRC Epidemiology Resource Centre, University of Southampton, United Kingdom;* [5]*Liggins Institute, University of Auckland, New Zealand.*

There is increasing evidence that early life nutritional constraint leads to altered epigenetic regulation of genes which persists throughout the life course leading to long term changes in metabolism and an altered susceptibility to disease. Studies from the Dutch Hunger Winter have shown differences in DNA methylation of a number of genes 60yrs after famine exposure (Tobi *et al.* 2009). Methylation levels, although initially thought to be stable, have been shown to change during the life course. We wanted to determine if methylation levels in the lipoprotein lipase (LPL) promoter (key enzyme in hydrolysis of triglycerides) vary over time and see whether methylation stability was affected by famine exposure.

Methylation status of 4 CpGs in the LPL promoter were investigated by bisulphite pyrosequencing in DNA isolated from peripheral blood samples from the same subjects 5yrs apart at age 58 and 63 (n=51) born as term singletons in Amsterdam, The Netherlands around the 1944-45 Dutch famine. Data were analysed by paired samples t-test and ANOVA.

There were no effects of famine exposure on the methylation level or methylation stability at any of the CpGs. Methylation stability was dependent upon sex at CpGs2 and 4. Methylation levels at CpG2 (-343 in regards to transcription start site; Chr8:19796763) were increased with age in men only ($p<0.05$, n=22). Methylation of CpG4 (-270) tended to decrease with age in women ($p<0.1$, n=29). CpG1 (-370) and CpG3 (-277) were stable over time in both sexes.

This study shows that some CpGs in the LPL promoter are more stable than others but that early life famine exposure does not affect this stability. We found that changes with age in single CpG methylation levels are sex specific. Understanding why specific CpGs change with time and how this is dependent upon sex will be important for the use of epigenetic marks in early life as predictive markers of future disease risk.

PI-051

Physical Activity and Cardiometabolic Risk Markers in Indian Children. A. Dhube[1], G.V. Krishnaveni[1], S.R. Veena[1], S. Kehoe[2], P. Coakley[2], C.H.D. Fall[2]. [1]*Epidemiology Research Unit, Holdsworth Memorial Hospital, Mysore, India;* [2]*MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom.*

To examine the association between accelerometer measured physical activity and cardiometabolic risk markers in children from a birth cohort in India.

Physical activity was measured using the Actigraph accelerometers (MTI AM7164 and GT1M) in 449 children between six and 10 years of age. Actigraph output was expressed as total counts. Time spent in different activity intensities was calculated based on counts per minute (cpm). Anthropometry, plasma glucose, insulin and lipid concentrations, and blood pressure (BP) were measured and insulin resistance was calculated (IR-HOMA) at 9.5 years of age. A questionnaire was administered to assess the routine activity pattern (for example: time spent in TV viewing, mode of transportation to school etc.) at the same time.

Total counts (4446547 v 376813) and cpm (573 v 487) were higher in boys compared to girls; girls had higher HOMA-IR at 9.5 years (0.6 v 0.9). After adjusting for sex and age at Actigraph measurement, total counts were negatively associated with 9.5-year percentage body fat (fat%; 29.6%, 27.5% and 25.5% from lowest to highest count tertiles, p=0.003). Higher total counts were associated with lower 30-minute plasma insulin concentrations at 9.5 years of age (254, 233 and 205 pmol/l, p=0.04; p=0.2 adjusted additionally for fat% and socio-economic status). Only in girls, time spent in vigorous activity (cpm>3000) was positively associated with plasma HDL-cholesterol concentrations (1.07, 1.07 and 1.09 mmol/l, p=0.046). Questionnaire data showed that children who spent more time watching TV during weekends had higher systolic (99.8, 101.4 and 102.5 mmHg, p=0.02) and diastolic BP (57.1, 58.4 and 59.6 mmHg, p=0.003). HOMA-IR was significantly lower in children who walked (0.7) or cycled (0.6) to school than those who used other mode of transportation (0.8, p=0.04). These associations were unchanged after adjusting for fat% and socio-economic status.

Using good quality data, we showed that higher physical activity is associated with better cardiovascular risk profile (lower fat% and higher HDL-cholesterol) in childhood. Questions about daily activity pattern complemented these findings. Lower insulin concentrations in association with higher physical activity may reflect improved insulin sensitivity in these children. The implication of these findings may become clearer with our continued follow-up.

PI-052

Dyslipidemia in Low Birth Weight Children in the Western Region of São Paulo: The Butantã Cohort. Maria Helena Valente[1], Filumena Maria S. Gomes[1], Leide Irislayne M. Araujo[1], Luis Marcelo I. Cirino[2], Isac de Castro[1], Alexandra Brentani[1], Ana Maria U. Escobar[1], Sandra Josefina F.E. Grisi[1]. [1]*Pediatrics, Faculdade de Medicina da Universidade de São Paulo, SP, Brazil;* [2]*Surgery, Faculdade de Medicina da Universidade de São Paulo, SP, Brazil.*

Introduction: Children with delayed intrauterine growth may have changes in body composition and present a higher risk of developing metabolic disorder. An association between low birth weight and subsequent high

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levels of blood cholesterol has been used to explain the hypothesis of the developmental origins of health and disease, emphasizing the relevance of fetal nutrition on adult disease.

Objective: To evaluate if there is a correlation between the lipid profile and low birth weight

A longitudinal and retrospective study of 41 low birth weight children followed at Butantã Cohort, from January 2007 to December 2009 was conducted. Butantã cohort is located in the western region of São Paulo City and is part of a research project of the Pediatrics Department. All neonatal period information was collected from hospital patient's records, and blood tests were performed in order to collect data on the lipid profile: total cholesterol (TC), HDL-cholesterol, LDL-cholesterol (LDL-c) and triglycerides (TG). Data about birth weight and lipid fractions were categorized and analyzed by the Mann-Whitney test for comparison of medians and quartiles.

Among low birth weight children, when evaluating the total cholesterol after one year of birth, we found that those who had high total cholesterol were born weighing 1.9 Kg (1.57 to 2.23) compared to those with normal total cholesterol, born weighing 2.3 Kg (2.2 to 2.4), resulting in a significant difference between medians p = 0.012. In this same category, when evaluating LDL cholesterol, we observed that children who had high LDL cholesterol had birth weight of 1.9 Kg (0.9 to 2.0) with p = 0.003. But when measuring triglyceride (TG) we found no significant differences (p = 0.21) among birth weight in children with increased TG 1.8 (1.6-2.2) as compared with those with normal TG 2.2 (1.8-2.4).

The increased total cholesterol levels and specially LDL –c were related the lower birth weight.

PI-053

Mechanisms Regulating Intrauterine and Embryonic Glucose Homeostasis. Sarah L. Finn-Sell[1], Ying C. Cheong[1], Roger Leandri[1], Tom P. Fleming[2], Nick S. Macklon[1], Judith J. Eckert[1]. *[1]School of Medicine, University of Southampton, United Kingdom; [2]School of Biological Sciences, University of Southampton, United Kingdom.*

Balanced glucose availability is critical for pre-implantation embryo development; however little is known about mechanisms controlling embryonic glucose exposure. In particular the regulation of the reproductive tract uterine fluid (UF), which provides glucose for the early embryo is poorly understood. This study used both mouse and human models to investigate how maternal glucose homeostasis may impact on embryo development.

Maternal serum samples from mice fed either a control (CT) or high fat (HF) diet for 3.5 days from plug were analysed for glucose and insulin (Sentinel Diagnostics assay, Mercodia elisa respectively n=10). Blastocysts collected by uterine flushing were examined for GLUT-1 or GLUT-3 protein localization using immunofluorescence and image analysis (metamorph n=18-24). Human serum and UF samples were analysed for glucose and insulin levels in relation to BMI as a surrogate measure for dietary habits (Normal BMI =18.5-24.9 n=13, Overweight BMI = 25.0-29.9 n=8, Obese BMI >30 n=2).

In the mouse a short term HF diet increases maternal serum insulin 2.91 fold ($p<0.01$) maintaining similar glucose levels compared to controls. Blastocysts from HF fed mothers had 21.4% and 52.5% more GLUT-1 and GLUT-3 localized to the membrane and reduced insulin stimulated glucose uptake ($p<0.05$).

In the human, glucose levels were 3.5 fold lower in UF than in serum ($p<0.01$) and were similar in all BMI groups. Obese women had 2.23-2.67 fold increased serum insulin levels compared to either normal or overweight women ($p<0.05$). There was no correlation between serum glucose or insulin and UF glucose.

An acute HF diet is sufficient to perturb maternal and embryonic insulin sensitivity in the mouse. Compensatory up-regulation of GLUTs in the embryo may be required to maintain glucose uptake in response to altered environmental stimuli. However, since BMI does not appear to impact on human UF glucose it is likely that mechanisms are in place to tightly control production and composition of these secretions, maintaining glucose concentrations at an optimum level. The novel concept of peri-implantation intra-uterine nutritional homeostasis requires further investigation and confirmation. Given the importance of balanced glucose metabolism to early embryo development, failure of regulation may have life long consequences.

PI-054

Impact of *Trans*-Fatty Acid Sources on the Fetal Programming of Atherosclerosis. Louise J. Gates[1], Jana Kraft[2], Simon C. Langley-Evans[1], Adam L. Lock[3], Andrew M. Salter[1]. *[1]Nutritional Sciences, University of Nottingham, Leicestershire, United Kingdom; [2]Animal Sciences, University of Vermont, VT, USA; [3]Animal Sciences, University of Michigan, MI, USA.*

Human epidemiological data and animal model studies clearly demonstrate that maternal diet can impact on the susceptibility of the offspring to atherosclerotic cardiovascular disease. While it is well established that dietary *trans* fatty acids (TFA) are associated with an atherogenic lipoprotein profile (raised low density lipoprotein and increased high density lipoprotein cholesterol), the impact of TFA in the maternal diet on development of atherosclerosis in the offspring has not been studied. Dietary TFA are primarily derived from Partially Hydrogenated Vegetable Oils (PHVO), though lesser amounts are also consumed within ruminant products (dairy & meat). These two sources provide very different isomeric profiles which may result in different bioactivities/biological effects.

Aim: To elucidate effects of maternal intake of different sources of TFA namely, PHVO and butter oil (BO, produced from TFA-enriched cow's milk), on the development of atherosclerosis in their offspring using the atherosclerosis-susceptible ApoE*3 Leiden mouse (AEL).

Female wild type C57BL/J6 mice were mated with heterozygous AEL males and fed diets supplemented with 13% either PHVO or BO. The fat sources were matched for total TFA (PHVO 16.5g; BO 16.9g/100g fat) but differed in TFA isomer profile. At birth dams were transferred onto a chow diet and female AEL offspring were subsequently weaned onto either a chow or atherogenic diet (13% cocoa butter, 0.2% cholesterol) for 12 weeks.

While plasma cholesterol was elevated in offspring fed the atherogenic diet ($p<0.001$), maternal diet, had no significant impact. Furthermore, while histological analysis of Oil Red O –stained sections through the aorta confirmed that while mice fed the atherogenic diet had more lesions than those fed chow ($p<0.0001$), there was again no difference between the offspring of dams fed the different TFA sources.

There is no differential effect of TFAs, derived from PHVO and dairy fat, in the maternal diet, on the development of atherosclerosis in the offspring.

PI-055

Simultaneous Determination of Genomic DNA Methylation and Uracil Misincorporation into DNA from Cells Exposed to Fumonisin B1 and/or Folate Deficiency. Afif M. Abdelnour, Céline Léridon, Frederic Tessier, Latifa Abdennebi-Najar, Abalo Chango, Diana Ringot. *SNES, Institut Polytechnique LaSalle Beauvais, France.*

Interest in folate metabolism has been growing this last decade, in part because of reports that link inadequate folate status to increased risk of diseases such as neural tube defects, preeclampsia and early pregnancy loss. Evidence from studies indicates that inadequate folate intake enhances the risk of cancer because folate has critical functions in biological methylation reactions such as DNA-5-cytosine methylation and in the synthesis of purines and a pyrimidine nucleoside (thymidine). Fumonisin B1 is a food contaminant, a mycotoxine that can also be teratogenic at least in part through interference with the utilization of folic acid, a dietary supplement used to reduce the incidence of neural tube defects. Fumonisin B1 interference with folate metabolism may result in genomic DNA hypomethylation, and excessive incorporation of uracil into DNA.

In order to study the effect of FB1 on genomic DNA methylation and uracil misincorporation into DNA we developed a HPLC/ESI-MS method. This sensitive new methodology allowed the quantification of the six bases contained in genomic DNA dU, dT, dG, dC, dA et 5-metC.

We have been able to quantify simultaneously DNA-5-methylcytosine and DNA-uracil into DNA. The linearity R2 coefficient between the MS signal and concentration of 5-metC in a range of 0.5 to 5µM or dU in a range of 10 to 100µM were 0.9954 and 0.9999 respectively. Results show that FB1 folate-deficient condition (1-100 µM) induced the hypomethylation of DNA-5-cytosine and the misincorporation of uracil into genomic DNA.

In conclusion, this HPLC/ESI-MS method allowing to measure 5-and uracil misincorporation into DNA may be suggested as an efficient approach for evaluation of DNA instability in folate deficiency.

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PI-056

Low B12 and High Folate: Novel Mechanism of Higher Insulin Resistance in Human Adipocytes. Antonysunil Adaikalakoteswari, Gyanendra Tripathi, Ponnusamy Saravanan. *Clinical sciences research institute, University of Warwick, United Kingdom.*

Prospective, longitudinal studies during pregnancy show low maternal vitamin B12 and high folate levels independently predict higher metabolic risk & insulin resistance in the offspring, higher rates of gestational diabetes and future type 2 diabetes to mother. It is intriguing that such B12/folate imbalance also causes hyperhomocysteinaemia, higher incidence of anaemia & cognitive dysfunction in elderly. As derangements in lipolysis have also been implicated in the pathogenesis of insulin resistance in obesity, we studied the molecular mechanisms of lipolysis in human adipocytes in various B12/folate conditions.

Human preadipocyte cell line Chub-S7 was grown and fully differentiated in different concentrations of B12 and folate: (1) Control (C): Normal B12 (0.5μM) + Normal folate (6μM); (2) Null (N): No B12 + No folate; (3) High Folate (F30): No B12 + folate (30μM) and (4) High Folate (F60): No B12 + folate (60μM) upto 21days. Glycerol release was measured for lipolysis. mRNA and protein expression were measured by qRT-PCR and western blotting respectively.

Glycerol release (μg/ml) significantly increased ($p<0.01$) in different conditions of B12/folate (N-19.6, F30-23.1, F60-22.7) compared to control (C-17.9). In addition, the mRNA levels (fold change relative to C vs N-0.35, F30-0.28, F60-0.35) and protein expression (fold change relative to C vs N-0.73, F30-0.89, F60-0.62) of perilipin, a lipid droplet coat protein was significantly ($p<0.05$) reduced. Lipases, ATGL (adipose triglyceride lipase) and HSL (hormone sensitive lipase), involved in lipolysis regulation were examined. Protein expression of ATGL (fold change relative to C vs N-0.73, F30-1.01, F60-1.30; $p<0.05$) was significantly increased but no change in HSL expression. Protein expression of metabolic regulator p-AMPK, (fold change relative to C vs N-0.86, F30-0.76, F60-0.53; $p<0.05$), and its downstream target p-ACC (acetyl coA carboxylase) (fold change relative to C vs N-0.87, F30-0.83, F60-0.69; $p<0.05$), which inhibits fatty acid oxidation were reduced in different conditions of B12/folate.

Our data demonstrate that B12/folate imbalance induces lipolysis and causes metabolic dysregulation by down-regulating AMPK activation. This novel mechanism may potentially be responsible for the insulin-resistance predisposition in individuals with B12 deficiency and high folate, a phenomenon increasingly common due to folic acid fortification programme.

PI-057

Altered Brain Neurotrophins at Birth: Consequence of Imbalance in Maternal Folic Acid and Vitamin B12 Metabolism. Sadhana R. Joshi, Pratiksha S. Sable, Kamini D. Dangat, Anvita A. Kale. *Nutritional Medicine, Interactive Research School for Health Affairs (IRSHA), Maharashtra, India.*

Folic acid and vitamin B12 are both vital constituents of the one carbon metabolism, which is speculated to mediate the cross talk between nutrition and complex regulatory mechanisms like epigenetics. We have earlier demonstrated in animals that DHA plays an important role in one carbon metabolism and influences global DNA methylation levels in the placenta. The alteration in one carbon metabolism with reduced DHA would lead altered levels and expression of important neurodevelopmental genes like Brain Derived Neurotrophic Factor (BDNF) and Nerve Growth Factor (NGF) since DHA is known to regulate levels of neurotrophins. The present study examines the effect of maternal folic acid supplementation at normal and excess levels both in the presence and absence of vitamin B12 on levels and expression of brain neurotrophins in Wistar Albino rats.

Pregnant female rats were assigned to six dietary groups with varying levels of folic acid and vitamin B12 (i.e. (NFB: 2mg folic acid+B12; NFBD: 2 mg folic acid – B12; EFB: 8 mg folic acid + B12; EFBD: 8 mg folic acid - B12; NFBDO: 2 mg folic acid – B12 + DHA and EFBDO: 8 mg folic acid – B12 + DHA)). On day 20 of gestation pup brain samples were collected to assess protein and mRNA levels of brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF).

Brain BDNF protein and mRNA levels were reduced ($p<0.01$ for both) in the EFBD group as compared to control. However, NGF protein levels were reduced ($p<0.05$) only in the EFBD group in comparison to EFB and control. Maternal supplementation of DHA improved pup brain NGF protein levels only in the NFBDO ($p<0.05$) and EFBDO ($p<0.05$) groups compared to NFBD and EFBD respectively.

Our results for the first time suggest that an imbalance in maternal micronutrients leads to a reduction in pup brain BDNF and NGF protein levels as well as their mRNA levels at birth. Maternal DHA supplementation to a micronutrient imbalanced diet could ameliorate the negative effects only for NGF but not for BDNF. Our findings are of relevance in view of the fact that regulation of neurotrophins like BDNF and NGF through early life environment may have stable and lifelong changes in brain and behavioral patterns.

PI-058

Maternal Vitamin B12 and Folate in Pregnancy Influence Offspring's One-Carbon Metabolism and Neurocognitive Development. H. G. Lubree[1], V. K. Bhate[1], P. A Katre[2], S. M. Joshi[1], D. S. Bhat[1], U. S. Deshmukh[1], N. S. Memane[1], S. R. Otiv[1], R. S. Ladkat[1], E. C. Rush[3], C. S. Yajnik[1]. *[1]Diabetes Unit, KEM Hospital Research Centre, Pune, India; [2]Persistent Systems P Ltd, India; [3]Centre for Child Health, Auckland University of Technology, Auckland, New Zealand.*

Vitamin B12 (B12) and folate are the key factors in one-carbon metabolism, cell division and cell growth. B12 deficiency is common in Indians. Maternal B12 and folate concentrations influence fetal growth and development. We investigated the relationship between maternal B12 and folate during pregnancy and offspring homocysteine concentrations and psychomotor development at 2y.

We measured B12 and folate concentrations in pregnant women attending antenatal clinics of the King Edward Memorial Hospital, Pune, India and related them to concentrations in the offspring at birth and 2y. We also measured child's homocysteine concentrations, and motor, mental and social development quotients (Developmental Assessment Scale for Indian Infants, and Vineland Social Maturity Scale). Breast feeding history was recorded. Demographic, body size and diet measurements for mother and child were made using standard methods.

One hundred and eighteen mothers (68 rural and 50 urban, 22.9 ± 3.9 y, BMI 22.8±3.0 kg/m^2) and their children (60 girls, 58 boys, birth weight 2.740±0.400 kg; 2y 10.3±1.2 kg) were studied. Pregnancy B12 concentrations (at 28 and 34 weeks) were low (70% were <150 pM), folate concentrations were normal to high (only one <7nM). Pregnancy B12 predicted both cord blood and 2y offspring concentrations while folate predicted only cord blood concentrations ($p<0.05$ for all). Child's 2y homocysteine concentration was high (11.4 ± 3.6 μM) and significantly predicted by lower pregnancy B12 (R^2 4.1%), lack of vitamin supplementation in pregnancy (R^2 5.6%), and if currently breastfed (R^2 8.4%, $p<0.05$ for all).At 2y, child's motor development was predicted by a higher pregnancy folate, while mental and social development was predicted by both B12 and folate ($p<0.05$ for all). Lower birth weight and higher head circumference predicted higher mental development quotients ($p<0.05$).

Low maternal B12 status in pregnancy and prolonged breast feeding contribute to high homocysteine concentrations in the child. Maternal B12 and folate in pregnancy also affect psychomotor development of the child. Pregnancy and lactation offer a unique window to influence child's one-carbon metabolism and brain development.

PI-059

Prenatal Maternal Mood Predicts Infant Hippocampal Structure and Function. Waseem Bak'r Hameed[1], Muhammad Farid Abdul-Rahman[2], Lit Wee Sim[2], Siti Aishah Bte Abdul Rahman[1], Shamini Sanmugam[1], Hui Jun Chong[1], Colin Hu[1], Helen Chen[3], Cornelia Chee[4], Yap Seng Chong[4], Seang Mei Saw[4], Kenneth Kwek[3], Keith Godfrey[5], Peter D. Gluckman[1,6], Michael J. Meaney[1,7], Marielle V. Fortier[3], Jen Richmond[8], Anqi Qiu[2], Anne Rifkin-Graboi[1]. *[1]Singapore Institute of Clinical Sciences, Singapore; [2]National University Singapore, Singapore; [3]KK Hospital, Singapore; [4]National University Hospital, Singapore; [5]University of Southampton, United Kingdom; [6]University of Auckland, New Zealand; [7]McGill University, Canada; [8]University of New South Wales, Australia.*

Maternal anxiety and depression predict children's affective illness and academic performance. Affective illness associates with the hippocampus,

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an area important to cognition. This is the first non-clinical examination of *relative* maternal mood upon human infant hippocampal structure and function.

At 26 weeks gestation, mothers enrolled in the Growing Up in Singapore towards Healthy Outcomes (GUSTO) cohort study completed the State Trait Anxiety Inventory (STAI, mean total score = 73.4, range 0-129, n = 734), Beck Depression Inventory II (BDI-II, mean = 8.7, range 0-46, n = 724), and Edinburgh Postnatal Depression Scale (EPDS, mean = 7.5, range = 0-23, n = 737). Mothers age at enrollment averaged 30.7 years (18-51, n = 1382). Infant neurocognitive outcomes included measures of neonatal axonal maturity (i.e., Fractional Anisotropy, "FA" via Diffusion Tensor Imaging), and six month memory performance (e.g., encoding speed during visual habituation, and observed recall during deferred imitation).

Preliminary results suggest after controlling for infant age, higher STAI scores predict and, respectively, marginally predict less FA in the right (B = -0.250, p = 0.034, n =71) and left (B = -0.207, p = 0.076, n = 72) neonatal hippocampus, and relate to less deferred imitation recall using logistic regression (B = -0.058, p = 0.026, n = 34). In addition, indices of depression (i.e., respectively BDI-II and EPDS) relate to less left hippocampal FA (B = -0.273, p = 0.028, n = 64) and longer habituation looking times, indicating less efficient encoding (e.g., B = 0.276, p = 0.039, n = 158).

Within a non-clinical population, relative differences in prenatal anxiety and depression may have lasting, and differential, impacts upon infant hippocampal development. This knowledge may aid intervention programs limiting school age cognitive and emotional problems.

PI-060

Dendritic Atrophy in Hippocampal CA3 Neurons in Parallel to Corticoid and Angiotensin Receptors Reduction in Adult Rats Submitted to *In Utero* Protein Restriction. Agnes Lopes[3], Daniele B. Torres[3], Ana J. Rodrigues[4], João J. Cerqueira[4], José M. Pêgo[4], Antonio F. Godinho[2], José A.R. Gontijo[3], Nuno Sousa[4], Patrícia A. Boer[1]. *[1]Department of Morphology, São Paulo State University, São Paulo/Botucatu, Brazil; [2]Toxicology Assistance Center, São Paulo State University, São Paulo/Botucatu, Brazil; [3]Department of Internal Medicine, State University of Campinas, São Paulo/Campinas, Brazil; [4]Life and Health Sciences Research Institute, University of Minho, Braga, Portugal.*

The aim of current work was to investigate the effects of intrauterine undernutrition in the adult hippocampal formation morphology and expression of mineralocorticoid (MR), glucocorticoid (GR) and angiotensina II (AT1) receptors.

Pregnant rats were divided into two groups: normal diet (NP, 17% of protein n=9) and low protein diet (LP, 6% of protein n=9). At 16 weeks of age brains were processed for 3D neuronal reconstruction in Golgi-Cox stained slices. For each selected neuron, all branches of the dendritic tree were reconstructed at 600X magnification using a motorized microscope attached to Neurolucida software. Fifteen neurons of regions CA1, CA3 and dentate gyrus (DG) were studied for each animal. Total length of trees and number of dendritic branches were compared across groups using 1-way analysis of variance. Other brains were processed for AT1, MR and GR immunohistochemistry (IH) (n=4) and for western blotting (WB) (n=4).

LP offspring presented a significant reduction in birth weight (LP 5.34 ± 0.06 vs NP 6.23 ± 0.05, n=95, p=0.0001). In adults, 3D analysis showed significant reductions in total length of CA3 basal, but not apical, dendrites (length: LP 920.51 ± 307.54µm vs NP 1264.72 ± 445.95µm, p=0.01). On the contrary, CA1 and DG dendritic arborizations were unaffected. By WB we verified that hipocampal expression of AT1 was 90% reduced in the LP group. IH revealed that GR expression was decreased in CA1 and CA3 hippocampal regions; no differences were found in MR expression.

Gestational protein restriction promoted significant length reduction of basal dendrites in hippocampal CA3 neurons in parallel with reduced GR expression in CA1 and CA3 areas and a massive reduction of AT1. Since the hippocampal formation is extremely vulnerable to both undernutrition and excessive corticosteroid concentration we suppose that elevated fetal exposure to maternal corticoids may be related with the genesis of these alterations.

FAPESP and CAPES supported this work

PI-061

A Transient Placental Source of Serotonin for the Fetal Forebrain. Alexandre Bonnin[1,2], Nick Goeden[1], Kevin Chen[3], Melissa Wilson[4], Jennifer King[4], Jean C. Shih[3], Randy D. Blakely[5], Evan S. Deneris[6], Pat Levitt[1,2]. *[1]Cell & Neurobiology, Keck School of Medicine of USC, CA, USA; [2]Zilkha Neurogenetic Institute, Keck School of Medicine of USC, CA, USA; [3]Pharmacology & Pharmaceutical Sciences, USC School of Pharmacy, CA, USA; [4]OB/GYN & Preventive Medicine, Keck School of Medicine of USC, CA, USA; [5]Pharmacology, Vanderbilt University Medical Center, TN, USA; [6]Neuroscience, Case Western Reserve University School of Medicine, OH, USA.*

Serotonin (5-HT) is thought to regulate neurodevelopmental processes through maternal-fetal interactions that have long-term mental health implications. A puzzling issue regarding the role of 5-HT in fetal brain development is that receptors, transporters and degrading enzymes for 5-HT often appear before the development of 5-HT innervation1 itself, suggesting the existence of an exogenous source of 5-HT at early stages of brain development. It is thought that beyond fetal 5-HT neurons, there are significant maternal contributions to fetal 5-HT during pregnancy, but this has not been tested empirically. Our goal was to examine putative central and peripheral sources of embryonic brain 5-HT.

To examine central and peripheral sources of fetal brain 5-HT, we used the Pet-1-/- mice in which most dorsal raphe neurons lack 5-HT. Using HPLC, we compared the concentration of 5-HT in embryonic brains harvested from Pet-1-/- and wild type littermates from embryonic day (E)10.5 to E17.5. We also used additional genetic strategies, a new technology for studying murine placental biology ex vivo, and direct manipulation of placental neosynthesis in vivo to investigate the nature of an exogenous source of 5-HT in the fetal brain.

Measures of 5-HT revealed previously unknown differences in accumulation between the fore- and hindbrain during early and late fetal stages, through an exogenous source of 5-HT. We show that this source is not of maternal origin. Results from in vivo, ex vivo and in vitro experiments revealed that this exogenous source is provided by a placental 5-HT synthetic pathway from a maternal tryptophan precursor, in both mice and humans.

This study reveals a new, direct role for placental metabolic pathways in modulating fetal brain development and indicates that maternal–placental–fetal interactions could underlie the pronounced impact of 5-HT on long-lasting mental health outcomes.

PI-062

Effects of Maternal Pre-Pregnancy Overweight, Obesity and Weight Gain during Pregnancy on Child Neurodevelopment in 2 Southern European Birth Cohorts. Maribel Casas[1,2], Anne-Elie Carsin[1,2], Pilar Amiano[3], Monica Guxens[1,2], Manolis Kogevinas[1,2,4], Katerina Koutra[5], Nerea Lertxundi[6], Mario Murcia[2,7], Marisa Rebagliato[2,7,8], Isolina Riaño[9], Clara Rodríguez[2,7], Theano Roumeliotaki[5], Jordi Sunyer[1,2], Leda Chatzi[5], Martine Vrijheid[1,2]. *[1]Centre for Research in Environmental Epidemiology/Hospital del Mar Research Institute, Spain; [2]Spanish Consortium for Research on Epidemiology and Public Health, Spain; [3]Public Health Division of Gipuzkoa, CIBER, Spain; [4]National School of Public Health, Greece; [5]Department of Social Medicine, University of Crete, Greece; [6]University of Basque Country/Basque Health Research Institute, Spain; [7]Centre of Public Health Research, Spain; [8]Public Health Board, Conselleria de Sanitat, Spain; [9]Servicio de Pediatria, Hospital San Agustín, Spain.*

Some epidemiological studies have shown that maternal pre-pregnancy overweight and obesity are negatively associated with offspring cognitive abilities. This study assessed whether maternal pre-pregnancy body mass index (BMI) and weight gain (WG) during pregnancy affects infant's neurodevelopment in two birth cohort studies (INMA-Spain and RHEA-Greece).

Pregnant women were recruited in four Spanish areas between 2004 and 2008. Maternal pre-pregnancy BMI and WG during pregnancy were classified according to WHO criteria. Child mental and psychomotor development was assessed around age 14m using the Bayley Scales of Infant Development. Scores were standardized by age. Multivariate linear regression models were adjusted for potential confounders.

Out of 1967 included mothers, 18.3% were overweight before pregnancy, 7.6% obese, whereas 23.7% and 37.4% of women were below or above their

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WG recommendation. After adjustment, maternal overweight and obesity were negatively associated with the child's psychomotor score (β=-1.81, 95%CI:-3.57; -0.05 and β=-1.77; 95%CI:-4.32; 0.78, respectively). Maternal obesity was negatively associated with mental score (β=-2.80; 95%CI:-5.35; -0.25). Lower and higher than recommended weight gain were associated with lower psychomotor scores (lower: β=-2.70, 95%CI:-4.47; -0.93 higher: β=-1.92, 95%CI:-3.48; -0.37), but not with mental scores.

Maternal pre-pregnancy overweight and obesity are associated with a reduction in psychomotor and mental scores at 14 months of age, and inadequate pregnancy WG with a reduction in psychomotor scores. This analysis will be replicated in 510 children from the RHEA cohort.

PI-063

Maternal Nutritional Effects on Hypothalamic Regulation of Pubertal Onset and Appetite in Prepubertal Females. K. L. Connor, M. H. Vickers, A. Bernal, D. M. Sloboda. *Liggins Institute & NRCGD, Auckland, New Zealand.*

We previously reported that a maternal high fat (HF) diet during pregnancy and lactation resulted in early pubertal onset, altered estrus cyclicity, hyperleptinaemia and obesity in adult offspring. We hypothesized that high leptin levels acting on the developing hypothalamus may mediate these long-term effects.

Wistar rats were fed a control (CON) or a HF diet (45% kcal as fat; HF) from conception to the end of lactation. In prepubertal females, brains were collected and hypothalamic nuclei from regions involved in satiety regulation and pubertal onset were punch dissected (MPOA: medial preoptic area; AVPV: anteroventral periventricular nucleus; ARC: arcuate nucleus; VMH: ventromedial hypothalamus). Protein and mRNA were extracted and mRNA levels measured by qPCR. ARC and VMH estradiol (E2) and leptin concentrations were determined by RIA and ELISA, respectively.

Female HF offspring had greater fat mass ($p<0.0001$) and plasma leptin levels ($p=0.01$) than CON. This was associated with decreased leptin levels in the ARC (2-fold) and VMH (1.8-fold) compared to CON, but changes were not statistically different. HF offspring ate fewer calories per gram of bodyweight from weaning until P25 than CON ($p<0.0001$). Preliminary data showed that HF females had a 1.1-fold decrease in ARC neuropeptide Y mRNA levels, associated with a 1.3-fold increase in proopiomelanocortin and a 2.6-fold decrease in somatostatin receptor 3 mRNA levels, although differences were not statistically significant. HF females tended to have lower ARC E2 levels (2.7-fold) and higher (1.3-fold) kisspeptin-1 receptor (GPR54) mRNA levels compared to CON. ERβ mRNA levels were 1.6-fold higher but GnRH mRNA levels 1.4-fold lower in the MPOA, although differences were not statistically significant. There were no differences between groups in genes examined in the AVPV.

Although preliminary, these data suggest that altered hypothalamic regulation of appetite may be present in prepubertal females born to HF fed mothers and this may act through the leptin receptor. Decreased ARC E2 levels in HF females may play a permissive role in the feedback regulation of GnRH, facilitating earlier pubertal onset through KISS-1 gene expression in the ARC and AVPV. Ongoing work will determine expression levels of ObRb and KISS-1 to establish central pathways through which leptin may contribute to altered appetite regulation and earlier pubertal onset in these offspring.

PI-064

Prenatal Glucocorticoid Treatment Influences Human Brain Development. Elysia Poggi Davis, Claudia Buss, L. Tugan Muftuler, Deborah A. Wing, Kevin Head. *Psychiatry, Pediatrics and Obstetrics and Gynecology, University of California, Irvine, CA, USA.*

Animal models indicate that prenatal exposure to excess glucocorticoids (GCs) has persisting consequences for brain structure and function. Although GC treatment is currently the standard of care for women in preterm labor, little is known about the implications for human neurodevelopment. During the 3rd trimester, the gestational period when GCs are given, these changes include formation of secondary and tertiary gyri, neuronal differentiation, dendritic arborization, axonal elongation, synapse formation and collateralization, and myelination. The rapidly developing fetal brain may be particularly susceptible to excess GCs. The objective of this study was to determine if prenatal GC treatment affects brain development among children who were full term at birth.

Brain development was evaluated using structural MRI in 54 six- to ten-year-old children born full term and recruited into two study groups (GC group:18 children prenatally treated with a single course of betamethasone; Comparison group: 36 children without GC treatment matched on gestational age at birth and sex). Cortical surface reconstruction was performed with FreeSurfer image analysis software suite. Comparisons were made to assess differences in cortical thickness. All analyses were False Discovery Rate (FDR) corrected for multiple comparisons.

Freesurfer analyses demonstrated regionally specific differences in cortical thickness between the study groups. Children exposed to prenatal GC treatment had a thinner cortex bilaterally in regions of the frontal cortex including the superior frontal lobe, the orbital prefrontal cortex and the precentral gyrus; the rostral and caudal anterior cingulate and the isthmus cingulate; the insula; and areas of the parietal lobe including the postcentral gyrus, the superior parietal lobule, and the supramarginal gyrus and the precuneus (p's <.05 FDR corrected).

Prenatal treatment with a single course of GCs was associated with a reduction in cortical thickness among children who were born at term. Confidence that this association is due to GC treatment is increased because participants were full term at birth and thus did not suffer from the well-documented effects of preterm delivery. These data suggest that prenatal GC treatment influences fetal neurodevelopment with consequences that persist through childhood.

This research was supported by NIH R01 HD050662.

PI-065

Weaned Rats Are More Susceptible to the Onset of Obesity Due to a High-Fat Diet. Luiz Felipe Barella, Júlio Cezar de Oliveira, Renato Chaves Souto Branco, Rosiane Aparecida Miranda, Clarice Gravena, Rosana Torrezan, Paulo Cezar de Freitas Mathias. *Department of Cell Biology and Genetics, State University of Maringá, Brazil.*

Obesity has spread to diverse populations worldwide. The current lifestyle of unbalanced diets coupled with sedentarism contributes to the development of metabolic syndrome. The peripubertal period in rats, which is characterized by several alterations, such as increased levels of steroid hormones, is critical for the final maturation of most neuroendocrine circuits, including those that regulate energy expenditure. The aim of this study was determine whether the introduction of a high-fat (HF) diet during the peripubertal phase induces significant changes in body weight control, glucose homeostasis and the parasympathetic tonus than when this diet is administrated to adult rats.

A HF diet was offered at weaning or during adulthood to male Wistar rats. The animals received the HF diet for 60 days. A group of animals received the HF diet for 60 days, from weaning to 81-day-old (HF81) or from 60 to 120-day-old (HF120), whereas two other groups received a normal-fat diet (NF81; NF120). Adiposity, glucose homeostasis, insulin sensibility and vagal activity were analyzed.

HF diet increased the accumulation of adipose tissue in all animals but to a much larger degree in animals fed a HF diet since weaning. The HF animals showed glucose intolerance (37% and 30% higher levels of glucose in HF81 and HF120 rats, respectively, $p<0.05$), with high levels of insulin secretion during the test ($p<0.05$). Rats fed a HF diet presented severe insulin resistance, as indicated by a low Kitt ($p<0.05$). These results are also dependent on the period that the diet was offered, and those animals that received a HF diet since weaning exhibited greater insulin resistance (47%) compared to those that received this diet in adult life (30%) ($p<0.05$). The recordings of vagus nerve activity showed that the HF rats had higher parasympathetic activity (HF81: 62%; HF120: 51%) than rats fed a normal-fat diet ($p<0.05$).

Our results show that a HF diet offered to rats both in the post-weaning and in adulthood causes impairment of glycemic homeostasis and an imbalance in parasympathetic activity. It is important to note, however, that a HF diet offered immediately after weaning has more drastic consequences than when administrated during adulthood.

PI-066

Neonatal Liver Fat and Adiposity: Hepatic Magnetic Resonance Spectroscopy in Offspring of Normal Weight and Obese Mothers. David E. Brumbaugh[1], Regina M. Reynolds[1], Mark S. Brown[2], Melanie Reece[1], Zhaoxing Pan[1], Jacob E. Friedman[1], Linda A. Barbour[3]. *[1]Pediatrics, University of Colorado, CO, USA; [2]Radiological Sciences, University of Colorado, CO, USA; [3]Medicine, University of Colorado, CO, USA.*

Both murine and non-human primate models have demonstrated that maternal high-fat diet leads to steatosis of the fetal liver. The relationship between maternal obesity and glucose intolerance and neonatal hepatic fat storage in humans has not been reported. We hypothesized that the burden of increased fat storage in neonates born to obese women with gestational diabetes mellitus (GDM) would increase hepatic lipid content compared to neonates born to normal weight mothers with normal glucose tolerance.

Obese mothers (pre-pregnancy mean BMI 40.1) with diet or medication-controlled GDM and normal weight mothers (pre-pregnancy mean BMI 21.8) with normal glucose tolerance were recruited two weeks prior to delivery. At 1-3 weeks of age, offspring of GDM mothers (n=9) and normal weight mothers (n=7) underwent air-displacement plethysmography (ADP) as well as magnetic resonance spectroscopy (MRS) of the liver utilizing a lipid standard for calibration. Hepatic fat was expressed as the ratio of the summed hepatic lipid peaks to the sum of lipid peaks in the lipid standard.

There was no significant difference in birth weight or total adiposity by ADP between infants groups. There was a non-significant trend (p=0.17) towards increased hepatic fat in infants born to GDM mothers (mean 0.0263, SD 0.0132) compared to normal weight mothers (mean 0.0189, SD .00743). There was a non-significant correlation between maternal pre-pregnancy BMI and hepatic fat (r=.38, p=.15) but little correlation between gestational weight gain and offspring hepatic fat (r=.032, p=.91). In all 16 infants studied, there was a non-significant correlation between increasing total adiposity, as measured by ADP, and increased hepatic fat (r=0.38, p=.14).

Utilizing a sensitive technique (MRS) for measurement of hepatic fat, in a small number of subjects our preliminary data suggest that increasing hepatic fat storage at birth may correlate with total adiposity in the neonate. Factors which drive fetal and infant fat accretion may increase the potentially detrimental lipid burden in the developing human fetal liver.

PI-067

Waist Height Ratio a Simple Indicator To Identify Obesity. Suneeta S. Chandorkar, Neha M. Vaidya, Ruchi Patel. *Foods & Nutrition, The M. S. University of Baroda, Vadodara, Gujarat, India.*

The present study was planned to map the regional prevalence of obesity, dyslipidemia, hs CRP and their interrelationship in adults (25-60years) in an urban University set up.

Subjects 25 - 60 years of age employed as research fellows or teaching faculty in the M. S. University of Baroda were enrolled for the study using random sampling technique. The final sample comprised of 292 subjects. Individuals on lipid lowering drugs, thyroid treatment and hormone replacement therapy were excluded from the study. Anthropometric measurements were taken using standard procedure. Fasting blood sample was collected for estimating lipid profile and hs-CRP levels of the subjects using diagnostic kit methods.

Eighteen percent of the subjects were overweight and 53% were found to be obese by Asia Pacific cut offs for BMI. Abdominal obesity in terms of WC, WHR and WhtR was found among 58%, 44% and 75% of the subjects respectively using gender wise appropriate cutoffs. High LDL and high TG were found among 26% and 19% of the subjects respectively while 51% had low HDL levels. hs CRP estimations put 38% of the subjects at higher risk of any kind of inflammation. Anthropometric indices except for WHR correlated significantly with lipid profile and hs CRP levels. However, no significant correlation was obtained between lipid profile and hs CRP. WHtR emerged as the best predictor of cardiovascular diseases followed by WC and BMI.

A large proportion of the study population was found to be at risk of cardio-metabolic disorders and appropriate interventions need to be planned for them. Waist Height Ratio emerged as a simple cost effective tool that can be used to screen large population.

PI-068

Maternal Overweight and Offspring's Metabolic Health in Adolescence in the Northern Finland Birth Cohort 1986. Shikta Das[1], Marika Kaakinen[2], Ulla Sovio[3], Sylvain Sebert[4], Jaana Laitinen[5], Anneli Pouta[6], Ann-Maj Samuelsson[7], Paul D. Taylor[7], Marjo-Riitta Jarvelin[1,2,6]. *[1]Imperial College London, United Kingdom; [2]Institute of Health Sciences & Biocenter, United Kingdom; [3]London School of Hygiene and Tropical Medicine, Finland; [4]University of Nottingham, United Kingdom; [5]Finnish Institute of Occupational Health, Finland; [6]National Institute of Health and Welfare, Finland; [7]Kings College London, United Kingdom.*

Evidence largely from animal studies suggests that maternal obesity during pregnancy may affect offspring's metabolic health. We investigated the impact of maternal obesity and it's interaction with body mass on adolescent's metabolic health.

We used the data from singletons in the Northern Finland Birth Cohort 1986 and divided offspring into two main groups by maternal pre-pregnancy body mass index (BMI): with normal-weight [NM, 18.5 < BMI < 25.0 kg/m2, n=4914] or overweight/obese [OM, BMI ≥ 25.0, n=1034] mothers. Each group was stratified into those who did or did not develop overweight/obesity (ovw/obe) by age 16 years (in total four groups) using the IOTF cut-offs for children. Offspring anthropometric data, blood pressure (BP), fasting serum glucose, insulin and lipids at 16 years were compared. Analyses were stratified by gender, adjusted for gestational age, Tanner puberty scale, age at menarche, variation in FTO gene (rs1421085) and maternal socio-economic status in a sequential manner to explore the impact of each factor.

28% of adolescents born to OM were ovw/obe compared to 13% of offspring of NM (P<0.001). The offspring of OM had more adverse metabolic values than offspring of NM even after adjustments. The ovw/obe adolescents born to OM had the highest waist-hip ratio (males only), BP (females), fasting insulin and generally the most adverse lipid levels compared to all other groups (P<0.001). However, ovw/obe adolescents of NM had also more adverse metabolic values (e.g. males: insulin 16.5 µmol/L) than normal weight adolescents either in NM (9.7 µmol/L) or OM (10.0 µmol/L) groups [the highest insulin in ovw/obe of OM, 20.3 µmol/L]. Although metabolic measures of normal weight offspring of OM were very similar to those of normal weight adolescent of NM they had higher BMI at 16y.

The study shows that maternal obesity is an important factor in the development of adolescent obesity which in turn determines the emergence of adverse metabolic profile.

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PI-069

Maternal Protein Restriction Influences Obesity Onset in Adult Rats. Júlio C. de Oliveira, Luiz F. Barella, Renato C.S. Branco, Rosiane A Miranda, Luiz A. Bataglini, Rosana Torrezan, Clarice Gravena, Paulo C.F. Mathias. *Department of Cell Biology and Genetics, State University of Maringá, Paraná, Brazil.*

Metabolic programming by early undernourishment leads to disruption in the food intake and energy controls. It has been observed that hypothalamic neurons density and function are changed in rats that were undernourished during uterine life, which allows to obesity onset when young or adult are exposed to high-caloric diet, such as hyperlipidic ones. Other potential windows to program metabolism, such as lactation period is less studied. This work aimed to study obesinogenic susceptibility of adult rats that underwent metabolic programming by maternal protein restriction during lactation.

Wistar dams rats received low-protein-diet (protein-4%) during the first 2/3 of lactation (LP-group) or normal-diet (protein-23%) during all lactation (NP-group). In the weaning, 21-day-old, the offspring of both groups received normal diet (LP and NP group) or high-fat diet (LP/HFD and NP/HFD group). Food intake and body weight (BW) gain were evaluated. After overnight fasting, blood samples from 90-day-old rats were collected by the jugular vein to evaluate fasting glycemia and insulinemia. After anesthesia (thiopental 45mg/kg BW) to decapitation, fat pads were removed and weighed.

The LP/HFD rats showed the higher magnitude of BW gain (277.0±1.7; LP/HFD *vs* 138.5±21.1; NP/FHD, p<0.001) and fasting insulinemia (361.0±36.9; LP/HFD *vs* 121.4±33.1; NP/HFD, p<0.001). The high-fat diet intake was 51.9% higher in the LP/HFD than NP/HFD (p<0.05). The

magnitude of retroperitoneal, epididymal, visceral and inguinal fat pads from LP/HFD rats were higher 15%, 43.7%, 35% and 56.7% respectively, than NP/HFD ($p<0.001$).
Results indicate that lactation protein restriction programs the metabolism to be sensitivity to high-fat diet-induced obesity.
Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES and Fundação Paranaense de Apoio à Pesquisa - Fundação Araucária.

PI-070

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

PI-071

Maternal Nutrition and Neonatal Adipocyte Function: Adiponectin and Retinol Binding Protein in Indian Neonates. Urmila Deshmukh[1], Suyog Joshi[1], Dattatray Bhat[1], Himangi Lubree[1], Lalita Ramdas[1], Klaus Kraemer[2], Chittaranjan Yajnik[1]. *[1]Diabetes Unit, King Edward Memorial Hospital and Research Centre, Pune, India; [2]Sight and Life, Basel, Switzerland.*
The susceptibility of Indians to type 2 diabetes is partly related to their higher adiposity, which is programmed in utero. We have reported effect of maternal nutrition on offspring adiposity, now we report its effect on offspring adipocyte function (i.e. circulating adipokines: adiponectin and retinol binding protein 4 (RBP4)).
In 221 pregnancies (106 urban, 115 rural), we studied maternal demography, anthropometry, diet and biochemistry (glucose, lipids, vitamin B12, folate, tHcy). Birth size was measured and cord blood was analysed for adiponectin (HMW) and RBP4 (n=148). Maternal vitamin A intake was assessed from frequency and portion size of vitamin A-rich foods consumed.
Mean (sd) age of the women was 23.2 (3.8) y, BMI (28wk) 22.2 (2.8) kg/m²; 32.4% were lacto-vegetarian and 34.5% consumed non-vegetarian food ≥ 4 times/mo.
The median (25th, 75th centile) cord adiponectin concentration was 13.1 (8.6, 18.9) µg/ml, there was no difference in urban vs rural, primi- vs multi-parous, pre-term vs full-term pregnancies, and in boys vs girls. Maternal size, her macronutrient intake (calorie, fat, protein) and circulating glucose and micronutrients were not associated, but maternal triglycerides, cord folate and neonatal length were positively associated with cord adiponectin. On MLRA, maternal triglycerides (R^2 3.9%), neonatal length (5.6%), and cord folate (2.4%) explained 12% of variation in cord adiponectin ($p<0.05$ for all).
Cord RBP4 concentration was 3.7 (2.7, 4.6) µg/ml, there was no difference between primi- vs multi-parous, preterm vs full term pregnancies. Cord RBP4 was higher in urban than in rural, and in girls compared to boys. Maternal intake of vitamin A-rich foods, neonatal abdominal circumference, sum of triceps and sub-scapular skin-folds, and cord folate had positive associations with cord RBP4. On MLRA, maternal intake of vitamin A-rich foods (R^2 4.1%) and neonatal sum of skin-folds (10.7%) explained a total of 14.8% of the variance in cord RBP4 ($p<0.05$ for all).
Our study indicates lower levels of adiponectin and RBP4 in Indian babies compared to westerners. Maternal nutrition, metabolism and neonatal body composition influence adipokines. Longitudinal follow up will help clarify role of maternal factors in programming of adipocyte function.

PI-072

Altered Adipocyte Structure and Function in Nutritionally Programmed Microswine Offspring. Elizabeth A. DuPriest[1,2,5,6], Philipp Kupfer[1,5], Baoyu Lin[1,5], Kaiu Sekiguchi[1,5], Terry K. Morgan[3], Kim E. Saunders[4], Tom T. Chatkupt[4], Jonathan Q. Purnell[1], Susan P. Bagby[1,2,5]. *[1]Medicine, Oregon Health & Science University, OR, USA; [2]Physiology & Pharmacology, Oregon Health & Science University, OR, USA; [3]Pathology, Oregon Health & Science University, OR, USA; [4]Comparative Medicine, Oregon Health & Science University, OR, USA; [5]Portland VA Medical Center, OR, USA; [6]Warner Pacific College, OR, USA.*
Adipose dysfunction links obesity with cardiometabolic dysfunction, but its role in developmentally-induced disorders is unknown. In 3-5 mo old non-obese juvenile microswine offspring exposed to perinatal maternal protein restriction (MPR) and exhibiting rapid adipose tissue accrual, we assessed markers typically associated with diet-induced or genetic obesity.
Adiponectin and TNF-α mRNA levels and adipocyte size were measured in intra-abdominal (ABD-AT) and subcutaneous (SC-AT) adipose tissue depots in juvenile offspring. Plasma cortisol, leptin, and insulin levels were measured in fetal, neonatal and juvenile offspring.
In juvenile low protein offspring (LPO), adipocyte size was reduced 22% in ABD-AT ($p=.011$), and female LPO had increased adipocyte size in SC-AT ($p=.05$), yet adiponectin mRNA was low in both sexes and in both depots ($p<.001$). TNF-α mRNA levels were unchanged. Plasma leptin ($p=.004$) and cortisol ($p<.05$) levels were reduced in neonatal LPO. Correlations between percent body fat and adiponectin or TNF-α mRNA, or plasma leptin levels were observed in NPO, but not LPO. In juveniles, plasma glucose was increased in male LPO, but decreased in female LPO (interaction, $p=.023$), but plasma insulin and insulin resistance were unaffected by MPR.
Findings support sex- and depot-specific programming of adipocyte size and function associated with early signs of altered glucose homeostasis. Since adiponectin and leptin dysfunction occur independently of obesity, adipocyte hypertrophy, or increased markers of inflammation, processes distinct from those in diet-induced and/or genetic obesity may be operative. Programmed adipose tissue dysfunction may constitute an etiologic link between adverse early nutritional environment and later chronic cardiometabolic disease.

PI-073

Preterm Placental Oxidative Stress and Reduced Placental Taurine Levels: Potential Marker of Increased Neurodevelopmental Risk? M. Alexander[1], E. Park[1], G. Schuller-Levis[1], I. Buhimschi[2], C. Buhimschi[2], C. Salafia[1,3]. *[1]Institute for Basic Research, Staten Island, NY, USA; [2]Yale University School of Medicine, New Haven, CT, USA; [3]Placental Analytics, LLC, Larchmont, NY, USA.*
Taurine is an essential amino acid which promotes healthy neurological development and is transported by the placenta. Placental taurine levels may be identical in normal first and third trimester placentas (Placenta 1994;15:747-751). Oxidative stress, a common correlate of preterm fetal growth restriction and preeclampsia, damages the placenta, potentially reducing placental taurine transfer. We test the hypothesis that placentas delivered under conditions associated with oxidative stress will have lower taurine levels than term controls.

80 total samples were studied. 16 healthy placentas from well grown infants without a maternal diagnosis of preeclampsia were compared to 64 preterm placentas obtained from cases of non-infectious PTL/PPROM, idiopathic abruption, severe preeclampsia, and/or fetal growth restriction between gestational ages 24-36 weeks. Taurine concentrations were determined in fresh frozen placental tissues by high-performance liquid chromatography. Statistical tests performed included ANOVA, regression and bivariate and partial correlations, with $p<0.05$ considered significant.

Mean taurine concentrations were 2.7 +0.1 mM, 3.3+0.1mM and 3.7+0.2mM in pregnancies delivered from 24-30 weeks, 30+-36 weeks, and term, respectively ($p<0.001$). Linear regression showed a strong a continuous relationship between placental taurine level and gestational age which was independent (each $p<0.0001$) of the amniotic fluid proteomic score and the specific clinical diagnosis type. After adjustment for amniotic fluid proteomic measures of inflammation and cord blood IL-6 levels, taurine remained significantly associated with gestational age ($p<0.0001$).

Our data are consistent with the hypothesis that placentas delivered preterm due to oxidative stress associated with non-infectious PTL/PPROM, abruption, severe preeclampsia and fetal growth restriction have reduced placental taurine levels. We speculate that these clinical conditions have reduced villous arborization and decreased distal capillary networks, and may carry increased risk for neurodevelopmental poor outcome in addition to effects of prematurity, at least in part due to aberrant placental-fetal taurine transfer.

PI-074

Preeclampsia Is Associated with Increased DNA Methylatiuon in Key Placental Genes. Cindy M. Anderson[1], Eric O. Uthus[2]. *[1]Family and Community Nursing, University of North Dakota, ND, USA; [2]USDA ARS, Grand Forks Human Nutrition Research Center, ND, USA.*

Preeclampsia (PE) affects 8-10% of women in the US and long-term consequences include subsequent development of maternal hypertension and hypertension in offspring. As methylation patterns are established during fetal life, we focused on epigenetic alterations in DNA methylation as a plausible explanation of the heritable development of hypertension resulting from maternal PE. In this proof-of-principle study our objective was to identify promoter regions of placental genes that were methylated only in PE.

Genome-wide CpG DNA methylation patterns were determined in placental tissue from women with normotensive pregnancy and PE (n=3/group) by using Human DNA Methylation 2.1M microarrays (NimbleGen). These arrays tile ~7000 bp upstream and ~3000 bp downstream from a promoter in 100 bp segments and cover ~28000 CpG islands. Criteria for gene selection included methylation within 1000 bp upstream or downstream of the respective gene's transcription start site. Raw data were analyzed using NimbleScan software. Promoters and genes with DNA methylation differences (hypo or hypermethylated) in PE vs normotensive samples were identified.

We identified 163 genes with DNA methylation present in placentas from pregancies complicated by PE and absent in placentas from normtensive pregnancies. The listing of genes was uploaded to DAVID (http://david.abcc.ncifcrf.gov) which provides functional interpretation of genes derived from genomic studies. We used a classification stringency of high to determine functional annotation clusters. Cluster 1 had an enrichment score of 5.59; this cluster contains the GO Terms of anatomical structure formation, angiogenesis, blood vessel development, vasculature development, and blood vessel morphogenesis. There are a total of 12 genes in this cluster; 10 of the 12 genes are common to all five GO Terms. Cluster 2 included a panel of metabolic genes (enrichment score 1.86). Cluster 3 included genes involved in apoptosis (enrichment score 1.77). Two genes involved in vitamin D metabolism were also differentially methylated in PE samples.

This work suggests that an epigenetic mechanism may be involved in PE. DNA hypermethylation in placental genes regulating blood vessel development and vitamin D metabolism from women with PE may underlie placental insufficiency associated with PE and program the fetus for future development of hypertension.

PI-075

Maternal Dietary Exposure during Pregnancy Induces Specific Changes in the Placental Transcriptome. Jane K. Cleal, Isabel E. Iwagboe, Mark A. Hanson, Graham C. Burdge, Karen A. Lillycrop. *Institute of Developmental Sciences, University of Southampton, United Kingdom.*

Feeding pregnant rats a nutrient restricted diet has been shown to have deleterious effects on placental development and induce an altered phenotype in the adult offspring. The aim of this study was to investigate the effects of maternal dietary restriction on the expression of the whole placental transcriptome in order to identify molecular processes involved.

Rats were fed one of three diets: control (C, 18% casein), protein restricted (PR, 9% casein) or 30% reduction in total food intake (UN) from conception. On day 18 of gestation dams were culled and the placentas collected (6 pooled placentas per dietary group). The placental transcriptome was analysed by Oxford Gene Technology (OGT, Oxford, UK) using an Agilent 14879 whole rat genome expression microarray (4 X 44K) G4131F (Agilent Technologies Inc., USA). Altered gene networks were identified using gene ontology analysis (GeneSifter™; www.genesifter.net; VizX Labs LLC, Seattle, USA). The results of the microarray analysis were confirmed for specific genes by real time RT-PCR.

2880 genes differed (>2-fold change) between placentas of PR and C dams (1349 increased, 1531 decreased). 3956 genes differed between placentas of UN and C dams (2303 increased, 1653 decreased). Gene ontology analysis showed that maternal PR induced changes (Z score >2) in the gene ontology pathways: response to stimuli, defence response and regulation of metabolic processes were decreased, while signalling and transport were increased. In contrast to placentas from PR pregnancies, maternal UN increased response to stimuli, defence response, regulation of metabolic processes as well as signalling and transport.

Our findings show that the effects of altered maternal diet on the expression of the placental transcriptome differ between dietary exposures. These changes highlight the potential ability of the placenta to respond to the maternal environment.

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PI-076

Withdrawn by Author

PI-077

Specific Association of Maternal Weight Change in the First Trimester of Pregnancy with Size at Birth. Ibrahima Diouf[1,2], Jérémie Botton[1], Olivier Morel[3], Monique Kaminsky[4,5], Marie-Aline Charles[1,2], Barbara Heude[1], Anne Forhan[1], The EDEN Study Group[1,2,3,4,5]. *[1]Unit 1018, Centre for Research in Epidemiology and Populations Health (CESP), Team 10 " Lifelong Epidemiology of obesity, diabetes and renal disease", INSERM, Villejuif, France; [2]Faculty of Medicine, University Paris-Sud, Kremlin-Bicêtre, France; [3]Obstetric and gynecology, Maternity of Nancy University Hospital, France; [4]UMRS 953, Epidemiological research on perinatal health and women's and children's health, INSERM, Villejuif, France; [5]University Pierre et Marie Curie, Paris, France.*

Maternal weight change during the first weeks of pregnancy is a component of periconceptional nutrition, but its specific role in fetal growth has not been fully explored in humans.

Our objectives were to investigate 1. The assocation between weight change in the first trimester of pregnancy (WCT1) and size at birth in term pregnancies, independently of later weight change in pregnancy; 2. The role of placental weight in this relationship.

From 2002 women included in the French EDEN study, 1744 mother-child pairs reached term, had prepregnancy weight available and at least five measures of weight in pregnancy. We extrapolated women's weight at each week of gestation with a three-degrees polynomial model and estimated weight change during each trimester of gestation.

We used a multivariate linear model to investigate the associations between WCT1 and birth size after taking into account potential confounders (age, parity, BMI, tobacco use, educational level, length of gestation and weight

change after the 1st trimester) and centre of study. Then, we performed path analyses to investigate whether the relation between WCT1 and birth size could be mediated by placental weight.
Median weight change in the first trimester of pregnancy was 3.2kg (interquartile interval: 1.9-4.6). After taking into account weight gain in later gestation, WCT1 was positively associated with birth weight (beta:17.7±4g/kg, p <0.0001, vs 27.4 g/kg for weight change in the 2nd trimester and 12.2 g/kg for weight change in the 3rd trimester) and with placental weight (4.8±1.5g/kg, p=0.001). Results of path analysis showed that there was no direct association between WCT1 and birth size but that this association was mediated by placental weight.
Weight change during the first weeks of pregnancy may impact on fetal growth independently of weight change later in pregnancy but through its effects on placental growth and function.

PI-078

Effect of Maternal Pregravid Body Mass Index on the Activity of Placental Cytochrome P4501A1. Barent DuBois[1,2], Perrie O'Tierney[3], Kent Thornburg[3], Ganesh Cherala[1,2]. *[1]College of Pharmacy, Oregon State University, OR, USA; [2]College of Pharmacy, Oregon Health & Science University, OR, USA; [3]School of Medicine, Oregon Health & Science University, OR, USA.*
Over 50% of women of child-bearing age are either obese or overweight. Obesity alters drug metabolism in the liver and kidney. However, the effect of maternal obesity on placental drug metabolism - an important line of defense for fetal exposure to xenobiotics and environmental pollutants- is unknown. Thus, we examined the effect of maternal pregravid body mass index (BMI) on the functional status of a select drug metabolizing enzyme, Cytochrome P4501A1 (CYP1A1).
Human term placental tissue was collected from uncomplicated pregnancies at OHSU. Placental cytosolic and microsomal fractions were prepared using differential centrifugation, and protein content measured using Bradford assay. The placental CYP1A1 activity was measured by incubating cytosolic or microsomal protein with a specific substrate (ethoxyresorufin) and NADPH regenerating system, and monitoring the formation of product (resorufin) at excitation/emission wavelengths of 530/590 nm. The placental CYP1A1 activity was compared between women with pregravid BMI <30 and BMI >30 using t-test (α=0.05).
The placental cytosolic CYP1A1 activity is significantly lower in the women with BMI >30 (142.0±17.4 fmoles/min/mg) than in the women with BMI <30 (180.0±44.3 fmoles/min/mg) (p<0.05). However, the placental microsomal CYP1A1 activity did not differ between the women of two BMI groups.
The placental CYP1A1 activity in the women with higher pregravid BMI is decreased significantly in cytosol fraction, whereas a trend towards a decreased activity in microsomal fraction was observed. These findings suggest that maternal obesity increases fetal exposure to xenobiotics and environmental pollutants.

PI-079

Rapid Uteroplacental Blood Flow in a Mouse Model of Intrauterine Growth Restriction. Antonio E. Frias, Jessica Hebert, Daogang Zha, Johnathan Lindner, Terry K. Morgan. *Oregon Health & Science University, USA.*
Intrauterine growth restriction (IUGR) is associated with placental pathology, which may be an underlying root cause of this common pregnancy complication. In addition, Doppler ultrasound of the fetal umbilical artery often reveals absent end-diastolic flow, which is a clinical sign of increased vascular resistance and uteroplacental insufficiency. These observations suggest that abnormal uteroplacental blood flow may play a significant role in IUGR.
We measured uteroplacental blood flow in pregnant day 15 transgenic mice (n=4) with three copies of the murine angiotensinogen (AGT) gene. This construct was designed to simulate a common human promoter variant associated with hypertension, preeclampsia, and IUGR. These mice had been bred into a C57BL/6 wild-type background for more than ten generations. We have recently shown they have IUGR pups with absent end-diastolic flow and placentas that over-express heat shock proteins and sflt-1 compared with controls. Blood flow volume and flux rates were measured in vivo in at least three pups per litter using microbubble-enhanced ultrasound imaging and compared with day 15 C57BL/6 controls (n=6). Virgin and maternal blood pressures were also measured by radio-telemetry. Differences between maternal genotypes was compared by ANOVA followed by Bonferroni/Dunn post-hoc testing.
Control animals had a virgin mean arterial blood pressure of 98 +/- 2 mmHg, which dropped to 83 +/- 5mmHg by day two of pregnancy. Transgenic mice had a virgin pressure of 103+/- 5mmHg and did not show normal pregnancy-induced reduction in pressure. Instead, they showed persistently elevated pressures (110 +/- 2mmHg) by day 15 (p<0.05). Placental blood flow flux rates were significantly different between genotypes (p<0.01). Controls reproducibly had a distinct spiral artery and showed slow filling of the placental labyrinth with a five second delay compared with the uterine wall. In contrast, transgenic mothers had no distinct spiral artery by ultrasound and showed nearly instantaneous filling of the labyrinth.
Our novel approach to real-time in vivo measurements of uteroplacental blood flow shows a faster flux rate through the placenta in this transgenic model of IUGR. Our preliminary analysis suggests increased maternal blood pressure alone cannot account for this faster rate of flow, but instead the answer may lie with differences in uterine spiral artery remodeling.

PI-080

Placental Size and Behavioural Disorders in Children and Adolescents. Natasha Khalife[1], Vivette Glover[1], Anna-Liisa Hartikainen[2], Anja Taanila[2,3], Irma Moilanen[3], Marjo-Riitta Jarvelin[1,2], Alina Rodriguez[1,4,5]. *[1]Imperial College London, United Kingdom; [2]University of Oulu, Finland; [3]University Hospital of Oulu, Finland; [4]Uppsala University, Sweden; [5]King's College London, United Kingdom.*
The role of the placenta in fetal programming is often a neglected area of study. We investigated placental size in relation to the development of behavioural disorders in youth at eight years and later at 16 years of age. We also examined sexual dimorphism due to sex differences in the placenta and behaviour.
Prospective data were obtained from The Northern Finland Birth Cohort (NFBC) 1986, which consists of 9479 children.
Mothers were recruited at the first antenatal visit and self-reported background information. Antenatal and birth data were obtained prospectively from medical records. Midwives measured placental weight and surface area according to standard procedures, within one hour after birth. Children were followed-up at eight and 16 years, when data on behaviour was collected respectively using the Rutter B2 and Strengths and Weaknesses of ADHD symptoms and Normal behaviour (SWAN) questionnaires.
We used regression analyses to investigate the association between placental size and behavioural outcomes, and controlled for birth weight, gestational age, socioeconomic factors and medical factors (including BMI).
There were significant positive associations between placental size (weight, surface area and placenta-to-birth-weight ratio) and behavioural problems in boys at eight and 16 years of age. Increased placental weight was linked with overall probable psychiatric disturbance (at 8y, OR=1.14, p=0.005), ADHD symptoms (inattention-hyperactivity at 16y, OR=1.19, p=0.024) and antisocial behaviour (at 8y, OR=1.14, p=0.014). No significant associations were detected among girls.
Placental overgrowth may occur as a compensatory mechanism to maintain fetal development in response to prenatal insults. However, such overgrowth has been associated with fetal wasting to provide amino acids for placental metabolism; this may encompass a potential mechanism whereby a large placenta can affect fetal development, ultimately leading to disease, including behavioural disorders. The observed effect in only boys may be due to the higher prevalence of behavioural problems among boys than girls, along with the understanding that male placentas may be more vulnerable to prenatal insults, and more readily undergo compensatory growth in response to such disturbances.

PI-081

Protein and Methyl Donors in Maternal Diet during Preconceptual Period, Gestation and Lactation Affect Fetal and Postnatal Growth, Energy Metabolism and Gene Expression in the Offspring. Valerie Amarger, Fanny Giudicelli, Patricia Parnet. *UMR 1280 Physiology of Nutritional Adaptations, INRA-Nantes University, France.*

Maternal nutrition during preconceptional period and gestation influences fetal growth and development and susceptibility to metabolic adult onset diseases, suggesting the existence of an early nutritional imprinting. Proteins and some amino-acids in particular clearly play a major role in these effects. Methionine is an indispensable amino-acid for protein synthesis and a key source of methyl groups for methylation reactions. It is involved in the folate cycle, a complex interaction between amino-acids, vitamins and minerals. An imbalance between these nutrients may dramatically affect fetal growth and development. Our objective is to identify how these nutrients influence fetal growth and energy metabolism in the short and the long term via a major epigenetic mechanism, DNA methylation, known to play an important role in nutritional imprinting.

We fed female rats diets restricted or not in proteins and supplemented or not with high levels of several nutrients from the folic acid cycle (methionine, folic acid, Vitamin B12, zinc, choline and betaine, further design as methyl donors or MD) during three weeks before mating, gestation and lactation. Dams fed diets restricted in proteins (PR) and/or supplemented in MD took less weight during pregnancy than control. Pups from dams supplemented in MD were significantly smaller, independently of the protein level, and pups from PR mothers not supplemented in MD were heavier than control pups at birth. Pups growth during lactation was severely reduced by PR in the maternal diet and this was amplified by MD supplementation. Homocysteinemia was highly increased in MD supplemented dams. Pups from PR dams show reduced levels of triglycerides and insulin at weaning. Pups from MD supplemented dams have two to four times less leptin that the unsupplemented ones. Expression of genes involved in fetal growth (Igf2, H19) and metabolism (PPARs) was affected by maternal diet.

MD supplementation strongly affects fetal growth probably through an impact on the regulation of key genes involved in resources allowance during gestation. Protein content in the maternal diet affects postnatal growth likely because of resources limitation. Metabolic effects suggest an impact of maternal diet on pancreas development and lipid metabolism in liver and/or adipose tissue.

PI-082

Is Prenatal Growth Associated with Body Composition in Later Life? Findings from a British Birth Cohort Study. David Bann[1], Rachel Cooper[1], Andrew Wills[1], Judith Adams[2], Diana Kuh[1], the NSHD Boneand Muscle Ageing Group[3]. *[1]MRC Unit for Lifelong Health and Ageing, Division of Population Health, University College London, London, United Kingdom; [2]Clinical Radiology, Manchester Royal Infirmary, Manchester, United Kingdom; [3]MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge, United Kingdom.*

Studies have reported positive associations between birth weight and lean mass in adolescence and adulthood. However, inconsistent findings have been reported between birth weight and fat mass, and few studies have been conducted in later adulthood. We aimed to examine the associations of birth weight with fat and lean mass measured in later adulthood (60-64 years).

Included were 531 participants aged 60-64 from the MRC National Survey of Health and Development who attended two out of the six regional clinics where data collection was completed 2006-11. Whole body fat and lean mass (excluding the head) were measured using dual energy X-ray absorptiometry. Potential confounders/mediators were childhood socioeconomic circumstances, indicated by father's occupational class at age 4, and adult height.

Birth weight was positively associated with lean mass in both sexes, with a modest increase in lean mass per 1kg increase in birth weight of 1.71 kg in men (95% CI: 0.24 to 3.17) and 2.42 kg in women (95% CI: 1.07 to 3.76). These associations remained after adjustment for childhood socioeconomic circumstances in both sexes, but were largely attenuated after adjustment for adult height in men (β per kg increase in birth weight: 0.35kg; 95% CI: -0.98 to 1.68), but not women (β: 1.29kg; 95% CI: 0.12 to 2.45). Birth weight was not associated with fat mass in either sex, before or after adjustments.

These findings, if confirmed in the full dataset (n=1700, available June), suggest that pre-natal growth affects lean mass, but not fat mass, in later adulthood. A high birth weight may reflect a greater number of muscle fibres attained at birth, which then track into adulthood. Attenuation of effect after adjustment for height suggests that this association is largely driven by increased body size in men, and additional factors in women. Optimal growth before birth may protect against the detrimental impacts of low muscle mass in later life.

PI-083

The Effect of Antenatal Factors and Postnatal Growth on Serum Adiponectin Levels in Children. Michael S. Boyne[1], Debbie S. Thompson[1], Clive Osmond[1,2], Carolyn Taylor-Bryan[1], Suzanne Soares-Wynter[1], Terrence E. Forrester[1]. *[1]Tropical Medicine Research Institute, The University of the West Indies, Mona, Jamaica; [2]MRC Life Course Epidemiology Unit, University of Southampton, Southampton, United Kingdom.*

A low level of serum adiponectin (hypoadiponectinaemia) is a marker of cardiometabolic risk in adults and children. We investigated whether antenatal factors and childhood growth are determinants of childhood adiponectin levels.

The Vulnerable Windows Cohort Study is an observational cohort of Jamaican mother/child pairs recruited during the antenatal period. Offspring anthropometry was measured at birth, at six weeks, three monthly to two years and then every six months. Bioelectrical impedance and phlebotomy for fasting glucose, insulin, lipids and adiponectin were done at mean age 11.5 years. Data on 176 girls and 146 boys were analyzed with age and sex- adjusted multivariate analyses. Growth intervals were defined as early infancy (0-6 months), late infancy (six months - two years) and childhood (2-8 years).

The size of mother, placenta, fetus and newborn were not significantly associated with adiponectin levels (P-values > 0.12). Current weight, BMI, body composition (i.e. lean mass, fat mass, % fat), waist circumference, glucose, insulin, insulin resistance (HOMA-IR), triglycerides and systolic blood pressure were inversely related to adiponectin (P-values <0.05). After adjusting for current BMI or waist, the associations of triglycerides and systolic blood pressure with adiponectin were not significant.

Faster growth during late infancy (in BMI) and childhood (in weight or BMI) were associated with lower adiponectin levels (P-values <0.05). Gains in height were not significantly related to adiponectin.

After adjusting for current waist circumference, faster growth during early infancy (either in weight [r= 0.13; p< 0.01] or BMI [r= 0.16; p< 0.01]) was positively associated with adiponectin. Faster growth during childhood in BMI (r= -0.31; p< 0.001) was inversely associated. These associations were similar after adjusting for HOMA-IR.

Maternal, fetal and newborn sizes are not determinants of adiponectin levels in Afro-Caribbean children. Faster rates of growth during infancy are associated with higher adiponectin levels while faster rates during childhood are associated with hypoadiponectinaemia. These associations were not mediated by current body size or insulin resistance.

PI-084

Ponderal Index Is Negatively Associated with the Proportion of Time Spent in Sedentary Activity among Rural Costa Rican Children Aged 6 to 12 Years. Tom D. Brutsaert[1], Daniel D. White[2], Fernandez M. Martin[1], Elizabeth A. Benevento[1], Kiersten Westbrook[2], Timothy B. Gage[2]. *[1]Exercise Science, Syracuse University, NY, USA; [2]Anthropology, University at Albany, SUNY, NY, USA.*

Regular physical activity has positive health benefits and there is an extensive literature on the social factors influencing physical activity patterns. However, little is known regarding early-life biological influences on later-life activity patterns. The aim of this project was to test the hypothesis that intrauterine growth restriction (IUGR) leads to increased sedentary behavior in childhood. Several previous studies have investigated this hypothesis in adolescents and adults but the results have been inconclusive. Previous studies have suffered limitations, including the validity of methods to assess physical activity, lack of consideration of maturation status in adolescents, and a failure to account for the dominant influence of social factors on the physical activity pattern.

Sixty 6-12 year old children, born to term, were recruited from a rural area of Costa Rica. Costa Rica offers a unique target population as children predominantly engage in free-play (i.e., their activity patterns are not overly managed by parents nor excessively structured by the built environment). Each child wore a GT3M Actigraph© accelerometer continuously over a 5-day period in order to assess the proportion of time spent in sedentary, light, moderate, or vigorous activity.

Boys spent less time in sedentary activity than girls, and both sexes increased sedentary time with increasing age. The ponderal index (g/cm3), as a marker of IUGR, was negatively associated with sedentary behavior, both as a main effect (P=0.044) and as an interaction with age (P=0.046). The association was stronger in younger children, and disappeared by age 10. Also, the effect-size was relatively small, with lower ponderal index children spending only 3% more of their day in sedentary activity, controlling for age.

Poor fetal growth may lead to decreased physical activity levels in childhood. However, these effects may be small and may diminish with age as social factors come to predominate in a child's physical activity landscape. If true, physical activity represents a pathway that could mediate (or be mediated by) the well-documented changes in body composition and muscle mass with IUGR, as these changes likely underlie some of the increased risk for adult chronic disease.

PI-085

Malnourished Neonates Detected by Body Composition Measurements Have Worse Health Morbidities Than Controls: A Population-Based Cohort Study. Angela E. Carberry[1,2], Camille Raynes-Greenow[2], Heather E. Jeffery[1,2]. *[1]RPA Newborn Care, Royal Prince Alfred Hospital, NSW, Australia; [2]Sydney School of Public Health, University of Sydney, NSW, Australia.*

Malnutrition contributes to more than half of under five child deaths in the world, of which the majority occur between the first day and week after birth. The major burden of neonatal malnutrition occurs at term gestation and is often poorly defined or recognized. When birthweight for gestational age is known neonatal malnutrition is described by Small for Gestational Age (SGA) and defined as < 10th percentile on population-based growth charts and Appropriate for Gestational Age (AGA) between the 10 - 90th percentile. The aim of this study was to assess body composition in newborns to better define the incidence of malnutrition and to determine if the health morbidities of these newborns were different to controls.

Eligible neonates were well term (≥ 37 weeks) neonates born within the area health service of RPA Hospital Sydney, Australia. The study period was between August and October 2010. Body composition measurements were undertaken within the first 48 hours after birth by air displacement plethysmography (PEA POD ®). Morbidity was defined by surrogate markers of substrate depletion: hypothermia (< 36.5°C, poor feeding (3 criteria), extended length of stay. The morbidity index included all three outcomes.

In the study period 782 neonates met the eligibility criteria and a total of 602 were measured (77 percent recruitment rate). Mean gestation of neonates was 39.49 ± 1.18 (mean ±SD) weeks and 52 percent were males. Percent body fat at birth differed between male and female infants and was 8.37 ± 4.29 and 10.03 ± 4.27 respectively. After sensitivity analysis, low fat was defined by ≤ 1 SD below the mean. The prevalence of AGA malnourished neonates was 7.3 percent, equal to approximately 335 of the 4783 term neonates born at RPA Hospital in 2010, who would otherwise remain unrecognized. Neonates with lower fat had a significantly worse morbidity index in comparison to neonates with normal fat percentages (p = 0.000, RR 4.4 (2.04, 9.49)).

This study is the first to define the hidden incidence of AGA malnourished neonates who are not identified using SGA on standard population-based growth charts. AGA malnourished neonates are underestimated and unrecognized. Infants with low fat have a significantly worse neonatal morbidity index than controls and are potentially at greater risk of early onset adult disease.

PI-086

Beta Hypertrophy, Hyperglycemia, Hyperinsulinemia and Hyperleptinemia in 3 Month Old Wistar Rat Offspring after Postnatal High Fat Programming. Marlon E. Cerf, Charna S. Chapman, Keith Williams, Johan Louw. *Diabetes Discovery Platform, South African Medical Research Council, Western Cape, South Africa.*

To determine the effects of maintenance on a high fat diet in utero and/or during postnatal life on body weight, circulating glucose, insulin and leptin concentrations, lipidemia and on alpha and beta cell size, number and volume in three month old rats.

Offspring were maintained on a high fat diet (40% fat as energy) during defined periods of fetal and postnatal life and studied at three months of age. The offspring studied were: HF1, HF2, HF3 and HFG (maintained on a high fat diet for either the first, second or third week of gestation or throughout gestation respectively), HFP (maintained on a high fat diet from birth to three months) and HFGP (maintained on a high fat diet throughout fetal and postnatal life). Control offspring were maintained on a standard laboratory diet (10% fat as energy). Body weight, circulating glucose, insulin, leptin, triglyceride and cholesterol (total, LDL and HDL) concentrations were determined in all offspring. Pancreata were harvested from HFG, HFP and HFGP offspring, formalin fixed and double immunostained for insulin and glucagon. Alpha and beta cell size, number and volume were assessed by image analysis.

HFGP offspring were heavier while HFP offspring were hyperglycemic and hyperinsulinemic compared to control offspring. Further HFP offspring were also hyperinsulinemic compared to HFG offspring. HFP and HFGP offspring were hyperleptinemic compared to control and HFG offspring. HFP offspring had greater alpha cell numbers compared to HFGP offspring with beta cell hypertrophy compared to the control, HFG and HFGP offspring. Further HFP offspring presented beta cell hypoplasia compared to HFGP offspring.

Postnatal high fat programming (from birth to three months) induced beta cell hypertrophy concomitant with hyperglycemia and hyperinsulinemia suggesting a beta cell compensatory response. Maintenance on a high fat diet throughout life resulted in heavier, hyperleptinemic offspring indicative of leptin resistance. The postnatal period from birth to early adulthood represents an extended critical period for high fat programming of beta cells and metabolic disease.

PI-087

Correlation of Birth Weight and Maternal Weight Gain during Pregnancy in a Nonhuman Primate Model of Intrauterine Growth Restriction. Keefe Chng[1], Louiza Chan[2], Yap Seng Chong[2], Robin Choo[1], Michael J. Meaney[1], Joanna D. Holbrook[1], Peter D. Gluckman[1]. *[1]Singapore Institute for Clinical Sciences, Singapore; [2]National University of Singapore, National University Health System, Singapore.*

A nonhuman primate model was established to study the maternal and neonatal outcomes of nutrition-mediated intrauterine growth restriction (IUGR).

Sexually mature macaques (Macaca fascicularis) were housed in groups of one male to three females to allow for natural breeding. Early pregnancy detection was achieved by regular ultrasound scans (GE Logiq S6) of female breeders. Detection of a gestational sac was used to indicate successful pregnancy. Measurement of the greatest length of the embryo was used to calculate the gestational age. After confirmation of pregnancy, dams were housed in individual cages in a screened roof compound with a daily temperature range of 26-32°C and constant humidity of 84%. Food intake was individually monitored and regulated throughout pregnancy. Pregnant dams were randomly assigned into IUGR or control cohorts. Control dams had access to ad libitum food (Laboratory Fiber-Plus® Monkey Diet 5049, LabDiet) throughout gestation, while nutrient-restricted IUGR animals were fed at 65% of control intake from gestational day (GD) 32 to GD 70, and 70% of control intake from GD 71 to the end of gestation GD 154-170.

Nutrient-restriction during gestation limits the weight gain of pregnant dams to = 7.4% of initial body weight, compared to control pregnant dams that gain significantly more weight = 27.5% (p<0.001). Both cohorts reach full term with the delivery of IUGR (n=12) neonates of significantly lower birth weight = 0.284 +/- 0.037 kg compared to control (n=13) neonates = 0.337+/- 0.025 kg (p<0.001). Furthermore, neonatal birth weights were found to be

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positively correlated with maternal weight gain, r = 0.6155 ($p<0.001$), and gestational length of IUGR animals was shorter = GD 159.1, compared to control animals = GD 163.5 ($p<0.001$).
We have established a nonhuman primate model of nutrition-mediated IUGR that induces low percentage weight gain of pregnant dams during gestation which correlates with full-term neonates of significantly lower birth weight compared to control dams.

PI-088

Weight and Thinness at Birth Are Associated with Symptoms of Polycystic Ovary Syndrome in Adulthood: Evidence for Two Programming Pathways. Michael J. Davies[1], Wendy A. March[1], Giles C. Lynne[1], Moore M. Vivienne[1,2]. *[1]Research Centre for the Early Origins of Health and Disease, Robinson Institute, University of Adelaide, South Australia, Australia; [2]Discipline of Public Health, The University of Adelaide, South Australia, Australia.*
Polycystic ovary syndrome (PCOS) is associated with metabolic impairment and reproductive disorders. The aetiology of PCOS is unknown and contested. Clinical features are consistent with perturbed development in fetal life but epidemiological findings have been inconclusive. We therefore aimed to examine potential fetal origins of PCOS.
We assembled a retrospective birth cohort of 948 singleton female babies born at one hospital in South Australia in 1973-75. Birth characteristics were obtained from hospital records and PCOS symptoms identified through interview and clinical examination when women were approximately 30 years old.
Based on the combination of PCOS symptoms, women formed seven outcome groups. Multinomial logistic regression analysis was used to investigate associations between birth characteristics and these outcome groups.
After adjusting for gestational age, two distinct birth characteristics were associated with two PCOS symptom groups. Each 100 g increase in body weight increased the risk of hyperandrogenism alone by 5% (relative risk ratio 1.05; 95%CI, 1.01 - 1.09). In contrast, each one unit increase in ponderal index decreased the risk of women having all three key PCOS symptoms (hyperandrogenism, menstrual dysfunction, and polycystic ovaries) by 21% (relative risk ratio 0.79; 95%CI, 0.66 - 0.93).
Two distinct birth characteristics were associated with different PCOS symptoms in adulthood. This demonstrates that PCOS is associated with fetal development, and suggests two programming pathways are operating. Our findings may explain disparities among previous studies examining this hypothesis and point to differing aetiologies for symptom clusters. This work will help direct decision making in the debate over symptoms that best represent the disorder.

PI-089

Infant Nutrition in Relation to Eating Behaviour and Fruit and Vegetable Intakes at Age 5. Lisanne Möller[1], Marieke de Hoog[1,2], Manon van Eijsden[2], Reinoud Gemke[3], Tanja Vrijkotte[1]. *[1]Public Health, Academic Medical Centre, Netherlands; [2]Epidemiology, Documentation and Health Promotion, Public Health Service Amsterdam, Netherlands; [3]Paediatrics, VU Medical Centre, Netherlands.*
Infant nutrition may influence eating behaviour and food preferences at older ages. The present study explored whether duration of breastfeeding and age at introduction of formula feeding and solid foods were associated with children's eating behaviour and fruit and vegetable intakes at age 5.
Data collection was part of the Amsterdam Born Children and their Development (ABCD) study, a prospective birth cohort in The Netherlands, and included 3624 children. During infancy, infant nutrition data were collected. Child eating behaviour (satiety responsiveness, enjoyment of food, slowness in eating and food responsiveness) was assessed by questionnaire (CEBQ) and fruit and vegetable intakes were calculated from a food frequency questionnaire, both filled in by the mothers when their child turned five.
Introducing solid foods after the age of six months was associated with less enjoyment of food (B: -0.07; 95% CI: -0.12, -0.01) and less food responsiveness (B: -0.04; 95% CI: -0.07, -0.01), whereas an introduction before four months was associated with less satiety responsiveness (B: -0.09; 95% CI: -0.16, -0.02), compared with an introduction at six months. Duration of breastfeeding and age at introduction of formula feeding were not clearly associated with later eating behaviour. Long duration of breastfeeding and late introduction of formula feeding were significantly associated with higher fruit and vegetable intakes. Introducing solid foods before the age of four months was associated with a higher fruit intake compared with an introduction at six months.
These findings suggest that longer duration of breastfeeding, delayed introduction of formula feeding and introduction of solid foods between four and six months may lead to healthy eating behaviour and food preferences at age 5.

PI-090

Parental Feeding Practices during the Weaning Period and Fruit or Vegetables Intake at 3 Years. Blandine de Lauzon-Guillain, Aisha Betoko Bapoma, Anne Forhan, Aline Charles. *UMR-S 1018, Team 10 'Lifelong epidemiology of diabetes, obesity and kidney diseases", INSERM, University Paris Sud 11, France.*
The British ALSPAC cohort highlighted that feeding home-cooked fruit or vegetable (FV) during the weaning period was related to increased FV intake at the age seven years whereas feeding ready-prepared FV was not. Moreover, the association between home-cooked vegetables and later vegetable intake was moderated by the age of introduction of home-cooked vegetables. The introduction of solid foods into the infant diet appears as a critical period in the development of food habits. The purpose was to examine whether parental feeding practices during the weaning period are related to fruit and vegetable intake at three years.
1296 children from the EDEN mother-child cohort were included in the analyses. Parental feeding practices were assessed by breastfeeding duration, age of introduction of several food groups, use of commercial fruit puree or vegetables dishes. Fruit and vegetable intakes at three years were assessed by a food frequency questionnaire. Early parental feeding practices were related to 3-yr fruit and vegetable intake, considered separately, by linear regressions.
Longer breastfeeding duration was related to higher 3-yr fruit and vegetable intake (all $p<0.001$). The median age of introduction of complementary foods was five months (interquartile range= 3-6 mo). Later introduction of any solid food was related to higher fruit or vegetable intake at three years (p for trend=0.004 for fruit and 0.06 for vegetables). The age of introduction of fruit was positively related to later fruit intake (p for trend=0.01). However, the age of introduction of vegetables was not associated with vegetable intake at 3y. Compared to children using commercial fruit purees at 24mo, those who never or used commercial fruit puree only before two years tended to have lower fruit intake at 3y (OR=0.7[0.5-0.9]) than the others. Finally, children who never consumed commercial vegetable dishes had higher vegetable intake at 3y than the others (OR=2.0[1.3-3.2]).
Parental feeding practices during the weaning period appeared to have a significant impact on later fruit and vegetable intake.
Funding: The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under the grant agreement n°FP7-245012-HabEat

PI-091

Associations between Newborn Characteristics, Eating Behavior in Infants Aged 4 Months and Post-Natal Growth. Blandine de Lauzon-Guillain[1], Jérémie Botton[1], Barbara Heude[1], Anne Forhan[1], Anne Chantry[1], Régis Hankard[2], Aline Charles[1]. *[1]UMR-S 1018, Team 10 'Lifelong epidemiology of diabetes, obesity and kidney diseases", INSERM, University Paris Sud 11, France; [2]CIC 0802, INSERM, Univesity Hospital of Poitiers, France.*
Rapid growth in infancy is considered to be a risk factor for overweight and obesity in childhood. Energy intake, but also sucking rate and infant appetite during early life, are associated with infant weight gain. The purpose was to examine whether birth weight and gestational age are related to infant eating behavior (EB) reported at four months and how eating behavior is associated with post-natal growth.
1075 infants from the EDEN mother-child cohort were included in the analyses. Infant's EB was assessed at age four months by maternal self-report (appetite, sucking intensity) and by food records (meal frequency, meal duration and, among exclusively bottle fed infants, milk intake). EB traits

were related to newborn characteristics and to growth from birth to 4 mo and from 4 to 8 mo by logistic or linear regressions adjusted for recruitment centre, infant's gender, infant's age at completion of food records, mode of feeding at the 4-month questionnaire and, for infant's growth analyses, parental BMI.

Pre-term infants were more likely to have at least one feed versus none during night (OR [95% CI]=1.9[1.1-3.3]) and to have longer feeds (18 additional min/day, p<0.0001) than full-term infants.

Among full-term infants, vigorous sucking throughout feed (OR=1.2[1.0-1.3]) and shorter duration of feeds (of 2 minutes/day on average, p=0.007) were related to higher gestational age. Infants with vigorous sucking (OR=1.5[1.1-2.0]), those considered as always hungry (OR=2.4[1.1-5.0]) and, among exclusively bottle-fed infants, those with higher milk intake (56 additional ml/day, p<0.001), had higher birth weight.

Infants with a vigorous sucking throughout feed at age four months, as well as, among exclusively bottle-fed infants, those with higher milk intake, had a faster weight growth from both birth to four months and four to eight months.

The association between gestational age and feeding ability, already known among pre-term infants, was highlighted among full-term infants. Some aspects of infant eating behavior, as assessed by simple questions to the mother, were related to prenatal conditions and were predictive of subsequent weight growth.

PI-092

Prevention of Accelerated Growth in Nutritionally Programmed Offspring Does Not Ameliorate Adipose Tissue Dysfunction. E. A. DuPriest[1,2,5,6], P. Kupfer[1,5], B. Lin[1,5], K. Sekiguchi[1,5], T. K. Morgan[3], K. E. Saunders[4], T. T. Chatkupt[4], J. Q. Purnell[1], S. P. Bagby[1,2,5]. *[1]Medicine, Oregon Health & Science University, OR, USA; [2]Physiology & Pharmacology, Oregon Health & Science University, OR, USA; [3]Pathology, Oregon Health & Science University, OR, USA; [4]Comparative Medicine, Oregon Health & Science University, OR, USA; [5]Portland VA Medical Center, OR, USA; [6]Warner Pacific College, OR, USA.*

Those exposed to adverse intrauterine environments have higher risk of cardiovascular disease. However, degree to which disease risk reflects early programming vs accelerated childhood growth is unknown. In miscroswine offspring exposed to maternal protein restriction (MPR), we recently found accelerated post-weaning growth, small adipocytes in intra-abdominal adipose tissue (ABD-AT), reduced adiponectin mRNA in ABD-AT and subcutaneous (SC)-AT, and disruptions in the normal relationships between % body fat and both leptin and adiponectin. In this study, we tested effects of post-weaning feed restriction on these outcomes.

In juvenile microswine offspring exposed to MPR from .75 gestation to 2wks lactation, then placed on *ad libitum* (AL) or feed restricted (FR) diet post-weaning, we examined the effect of FR on growth, body composition, and adipose tissue function in Low Protein Offspring (LPO).

Accelerated growth was prevented by post-weaning FR; both Normal Protein Offspring (NPO) and Low Protein Offspring (LPO) had similarly reduced growth in response to FR. At 11wks, lean mass was reduced in all FR, and % body fat was low only in female LPO-FR. Reduced ABD-AT adipocyte size observed in LPO-AL was not corrected by FR. Similarly, reduced adiponectin mRNA levels in ABD-AT and SC-AT observed in LPO-AL were not corrected by FR. FR failed to restore relationships between % body fat and both leptin and adiponectin in LPO. FR lowered plasma glucose levels in NPO, but not in LPO; however, insulin sensitivity (assessed by QUICKI index) was increased by FR in both NPO and LPO.

Our data demonstrate that the altered adipocyte structure in Ad Lib LPO, the low adiponectin mRNA, and disordered relationships of adipokines with body fat are not consequences of accelerated post-weaning growth. Thus, these abnormalities of adipocyte structure and function likely reflect other pathways of perinatal nutritional programming.

PI-093

Growth Rate in Early Infancy and Body Fat Distribution in Healthy 5 Year Old Children. Annemieke M.V. Evelein[1], Frank L.J. Visseren[2], Cornelis K. van der Ent[3], Diederick E. Grobbee[1], Cuno S.P.M. Uiterwaal[1]. *[1]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Netherlands; [2]Department of Vascular Medicine, University Medical Center Utrecht, Netherlands; [3]Department of Pediatric Pulmonology, Wilhelmina Children's Hospital, University Medical Center Utrecht, Netherlands.*

Rapid growth in infancy and visceral adiposity are both associated with cardiovascular disease risk. Although increased childhood weight gain is related to central adiposity later in life, the contribution of early postnatal growth on abdominal fat distribution in children remains unclear. The aim of this study was to determine the relation between early postnatal growth and abdominal fat distribution at five years of age.

The ongoing WHeezing Illnesses STudy LEidsche Rijn (WHISTLER) birth cohort was used to obtain monthly growth data from birth to three months of age. In the first 378 children who turned five years of age, ultrasonographic measurements of the abdomen were performed to obtain measures of intra-abdominal and subcutaneous fat. Individual growth rates were assessed by linear mixed modelling allowing for random slope and intercept, followed by linear regression modelling stratified by child on the predicted weight and length values.

Postnatal weight for length gain (WfLG) was positively related to intra-abdominal and subcutaneous fat. Per one standard deviation (SD) increase in WfLG intra-abdominal and subcutaneous fat were 0.68 mm (95%-confidence interval (CI) -0.014 – 1.4; p-value 0.05) and 0.42 mm (95%-CI 0.063 – 0.77; p-value 0.02) higher respectively, adjusted for age, gender, current height and observer. To study relative differences in fat compartments, analyses were repeated using Z-scores; per one SD increase in WfLG Z-score intra-abdominal and Z-score subcutaneous fat were 0.12 SD (95%-CI -0.002 – 0.25; p-value 0.05) and 0.13 SD (95%-CI 0.019 – 0.24; p-value 0.02) higher respectively. These associations were not modified by birth size.

Variations in growth pattern in the first three postnatal months are related to differences in fat distribution in five year old healthy children. Over the whole range of birth size, higher weight for length gain is associated with an adverse fat distribution, characterized by more subcutaneous fat and a proportionally larger intra-abdominal fat compartment. These results give more insight in the development of differences in fat distribution, with possible metabolic consequences later in life.

PI-094

Growth Rate in Early Infancy and Vascular Properties in Healthy 5 Year Old Children. Annemieke M.V. Evelein[1], Frank L.J. Visseren[2], Cornelis K. van der Ent[3], Diederick E. Grobbee[1], Cuno S.P.M. Uiterwaal[1]. *[1]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Netherlands; [2]Department of Vascular Medicine, University Medical Center Utrecht, Netherlands; [3]Department of Pediatric Pulmonology, Wilhelmina Children's Hospital, University Medical Center Utrecht, Netherlands.*

Early life growth pattern has been shown related to later cardiovascular disease (CVD). Although differences in CVD risk factors have been determined even in childhood, it remains unknown whether rapid infancy growth is related to pre-atherosclerotic changes in young children as well. The aim was to study the relation between early postnatal growth and vascular properties in early childhood.

We used the ongoing WHeezing Illnesses STudy LEidsche Rijn (WHISTLER) birth cohort to obtain monthly growth data from birth to three months of age. In the first 296 children who turned five years of age, ultrasonographic measurements of the carotid artery were performed to obtain carotid intima media thickness (CIMT), distensibility and Elastic Modulus (EM). Individual growth rates were assessed by linear mixed modelling allowing for random slope and intercept, followed by linear regression modelling stratified by child on the predicted weight and length values.

Birth size and postnatal weight for length gain (WfLG) were not associated with vascular properties when analyzed separately. However, birth size modified the associations between WfLG and both distensibility and EM significantly; p values for interaction were 0.041 and 0.032 respectively. In children born relatively thin, per one standard deviation (SD) increase

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in Z-score WfLG, distensibility was 3.1 MPa^{-1} (95% - confidence interval (CI): -8.5 – 2.2) lower and EM was 5.2 kPa (95%-CI: -4.6 – 15.1) higher. In children born relatively thick, distensibility was 2.1 MPa^{-1} (-3.2 – 7.3) higher and EM 5.2 kPa (-13.6 – 3.1) lower respectively per one SD increase in Z-score WfLG (adjusted for age, gender, current height and observer). No significant interaction for CIMT was present.

Variations in growth pattern are not only associated with differences in CVD risk factors, but are related to changes in vascular function in early childhood as well. The thinner children are born, the stiffer are the arteries at five years of age with increasing weight for length gain in the first three postnatal months. These results contribute to a better understanding of the increased CVD risk associated with different growth patterns.

PI-095

Does Birth Weight Influence Physical Fitness in Stunting and Overweight Children? Marcos Galván[1], Guadalupe López[1], Viridiana Pérez[1], Lorena Fernández[1], Ricardo Uauy[2]. *[1]Institute of Health Sciences, U. Autonoma de Hidalgo, Pachuca, Mexico; [2]Institute of Nutrition and Food Technology, U. of Chile, Santiago, Chile.*

To evaluate in Mexican school children the relationship among birth weight, nutritional status and physical fitness controlling the effect of relevant confounder.

In a representative sample of 600 children from Hidalgo, Mexico, enrolled in public and private schools of first, third and sixth basic grade in 2010, we obtained birth weight (BW) and maternal age from official registries. At 8.9 ± 2.1 years, we evaluated physical performance (PhP) in children with six minute walk test (6MWT), weight and height by trained personal. Stunting (ST) was defined -<2 Z height for age (HAZ), no-stunting (N-ST) ≥-2 HAZ, overweight (OW) > +1 Z BMI for age (BAZ) and no-overweight (N-OW) < + 1 to ≥ -2 BAZ (WHO 2007). Variables were analysed as continuous and categorical; non-parametric statistics and multivariate analysis were done. 48.4% were girls, average BW was 2880.8 ± 679.8 g in ST and 3169.2 ± 574.5 g in N-ST ($p<0.001$), 3292.8 ± 634.7 g in OW and 3089.1 ± 563.4 in N-OW ($p<0.001$). BW categories were: 14.1% 1000 to 2500 g (BW1), 28.3% >2500 to 3000 g (BW2), 54.6% >3000 to 4000 g (BW3) and 2.8% >4000 g (BW4). In overall the 6MWT was 481.2 ± 79.5 m, with differences by nutritional status. Linear regression showed that these associations were stronger; BW explained 17% (R^2) of variability in 6MWT. Interaction between BW and nutritional status was tested ($p<0.05$). Adjusted models (sex and age of children, maternal age at birth and school type) of 6MWT for all BW categories and nutritional status showed lower PhP in stunted infants [302.5 ± 69.4 m BW1, 300.0 ± 70.5 m BW2, 301.4 ± 71.7 m BW3, 331.4 ± 95.1 m BW4 ($p>0.05$)]. Non-stunted and overweigh children in all categories showed higher PhP [N-ST: 371.7 ± 20.9 m BW1, 375.2 ± 19.8 m BW2, 376.3 ± 19.4 m BW3, 420.2 ± 27.7 m BW4 ($p<0.02$); OW: 383.6 ± 28.4 m BW1, 378.4 ± 24.1 BW2, 387.9 ± 22.9 BW3, 446.9 ± 40.6 BW4 ($p<0.02$)]. In BW4 children, the nutritional status effect on PhP was stronger than all BW categories.

This study provides evidence that the chronic nutritional deficits as reflected by stunted growth eliminate the protective effect of high birth weight on physical performance at school age. The relationship between BW and PhP is dependent on nutritional status at school age; finally a high birth weight condition better physical performance in non-stunted and overweight children.

PI-096

Effects of Antenatal Betamethasone Treatment on Anthropometrics and Neonatal Outcome in Male Twins. Hannah C. Gil[1], Deborah M. Sloboda[2], Boris Tutschek[3], Joachim W. Dudenhausen[1], Andreas Plagemann[1], Ernst J. Beinder[4], Thorsten Braun[1,4]. *[1]Departement of Obstetrics, Charité Campus Virchow, Germany; [2]The Liggins Institute University of Auckland and The National Research Centre for Growth and Development, New Zealand; [3]Department of Obstetrics and Gynecology, Inselspital Bern, Switzerland; [4]Division of Perinatal Programming Charité Campus Virchow, Germany.*

We have recently shown that in singleton pregnancies antenatal glucocorticoid treatment was associated with impaired fetal growth and neonatal outcome. However, there are currently no data analyzing this relationship in twin pregnancies. The aim of this study was to investigate the effects of antenatal betamethasone(BET) treatment on anthropometrics and neonatal outcome first in male twins.

Effects of maternal BET on neonatal anthropometrics, placental weight, cord blood gases and Apgar scores were collected retrospectively from pregnancy and birth data (1996-2011) and analysed independent of dose. BET-treated women were compared to gestational age-matched controls(663 male-male twin pairs: control=462, BET=864). Significance was accepted for $p<0.05$.

Male twins live-born between 23-25 weeks of gestation (wks) had a significantly higher Apgar-1 minute score after BET treatment compared to controls. BET was associated with sig. lower birth length (-5%), birth weight-to-placenta weight ratio ("placental efficiency" -12%), and ponderal index (-10%) between 26-28 wks; sig. lower birth weight centiles (-10,67 centiles) between 32-34 wks; and sig. lower birth weight (-4%), birth length (-2%) and placenta weight (-5%) between 35-37 wks compared to controls at each timepoint. In contrast in BET treated twins placental efficiency was sig. increased at 37 wks (+5%). BET was associated with an increase in mean head circumference (+1%) between 38-40 wks compared to controls. These changes were independent of the time of treatment or the time of pregnancy after BET treatment. 36% percent of BET-treated male twins were delivered after 34+0 wks.

In male twins born between 23-25 wks, BET was associated with higher Apgar scores: this effect was less in neonates born later in gestation. In BET, birth weight and body length were decreased and resulted in a symmetrical growth restriction, associated with a compensatory increase in placental efficiency later in pregnancy. Further analysis of within-twin pair and between twin-pair associations are ongoing to eliminate environmental and genetic factors.

PI-097

Preterm Birth and Premenstrual Symptoms in Adult Life – The Helsinki Study of Very Low Birth Weight Adults. Sanna Mustaniemi[1,2], Tia Aalto-Viljakainen[3,4], Marika Sipola-Leppänen[5], Petteri Hovi[3,6], Uriel Halbreich[7], Marja Vääräsmäki[1,2], Katri Räikkönen[8], Anu-Katriina Pesonen[6,8], Kati Heinonen[8], Anna-Liisa Järvenpää[6], Johan G. Eriksson[3], Sture Andersson[6], Eero Kajantie[3,6]. *[1]Child and Adolescent Health and Welfare Unit, National Institute for Health and Welfare, Oulu, Finland; [2]Department of Obstetrics and Gynaecology, Oulu University Hospital, Oulu, Finland; [3]Diabetes Prevention Unit, National Institute for Health and Welfare, Helsinki, Finland; [4]Department of Obstetrics and Gynaecology, Helsinki University Central Hospital, Helsinki, Finland; [5]Diabetes Prevention Unit, National Institute for Health and Welfare, Oulu, Finland; [6]Children's Hospital, Helsinki University Central Hospital, Helsinki, Finland; [7]Biobehavioral Program, School of Medicine & Biomedical Sciences, Buffalo, NY, USA; [8]Department of Behavioural Sciences, University of Helsinki, Helsinki, Finland.*

Premenstrual syndrome (PMS), premenstural dysphoric disorder (PMDD) and related symptoms cause a significant public health burden, for example the impairment and lowered quality of life for PMDD approaches the level of major depressive disorder. The origins of these conditions remain poorly understood. Hormonal systems such as glucocorticoid metabolism may be involved. As these systems may be programmed early in life, we hypothesized that young adult women born at very low birth weight (VLBW; < 1500g) have more PMS symptoms than their peers born at term.

We compared 75 VLBW women with 95 women born at term, all from the Helsinki Study of Very Low Birth Weight Adults. We used a validated, widely used questionnaire to assess self-reports of Menstrually-Related Disorders. The symptom scores were used both as continuous and as dichotomized variables. Multiple linear and logistic regression was used to adjust for confounders.

There was no difference in the continuous symptom score before menses (mean difference VLBW-term -18.3%, 95% confidence interval -37.9 to 7.5%) or after menses. The prevalences of premenstrual symptoms causing severe impairment to daily life were 13.3% for VLBW women and 14.7% for control women. For PMDD, they were 8.0% and 4.2%, and for PMS, 12.0% and 11.6%, respectively. These differences were not statistically significant ($p>0.1$).

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These findings suggest that the severity of premenstrual symptoms and the prevalence of PMDD and PMS among young women born at preterm and VLBW is not higher than among their peers born at term.

PI-098

Preterm Birth and Gestational Age, but Not Antenatal Corticosteroids, Alter Cardiac Autonomic Activity in Adult Sheep. Mary J. Berry[1], Anne L. Jaquiery[1,2], Mark H. Oliver[1,2], Jane E. Harding[1,2], Frank H. Bloomfield[1,2]. *[1]Liggins Institute, University of Auckland, Auckland, New Zealand; [2]National Research Centre for Growth and Development, New Zealand.*

Babies born preterm are also born small and are often exposed to antenatal corticosteroids. Increased cardiovascular risk in adults is predicted by increased cardiac sympathetic activity or parasympathetic withdrawal. Heart rate variability (HRV) is a means of assessing these aspects of cardiac autonomic function. We hypothesized that preterm birth in lambs is associated with changes in autonomic activity consistent with increased cardiovascular risk, and that this is independent of antenatal corticosteroid exposure.We aimed to determine the effects of preterm birth, gestation length and antenatal corticosteroid exposure on HRV in sheep.

Singleton bearing ewes were randomized to corticosteroid-induced delivery at preterm (Prem; 137 (137-138) days gestation [median (range)] male n=19, female n=13) or term (T-Dex; 147 (146-151)d, male n=15, female n=16) or to spontaneous term delivery (T-Spont; 147 (142-156)d, male n=12, female n=16). Birthweight (bwt) z-scores and weight for length [weight (kg)/(crown rump length+hind limb length (m))] at 16 months of age were calculated. Resting ECGs were analysed for HRV indices of sympathetic activity (LFnu), sympatho-vagal tone (LF/HF) and parasympathetic activity (SDΔNN, NN50%). Non-parametric data were log transformed. Data (mean±SEM) were analysed by ANOVA with Tukey post hoc test, adjusting for bwt z-score, age and weight-for-length.

Prem males had increased sympathetic but lower parasympathetic indices than T-Spont males (Prem vs T-Spont (all $p<0.05$); LFnu: 65±5 vs 48±5; Log LF/HF: 1.8±0.1 vs 1.4±0.1; Log SDΔNN: 2.8±0.2 vs 3.4±0.2; Log NN50%: 65±5 vs 48±5). There were no differences among T-Dex males and Prem or T-Spont males or among females in any group. In T-Spont females gestational age was negatively associated with LFnu and LF/HF (LFnu -4.3±1.6 units/day increase in gestational age (GA); $R^2=0.7$, $p=0.01$; LF/HF -0.06±0.03/d increase in GA; $R^2=0.8$, $p=0.04$). There was no association between bwt and HRV indices.

Preterm birth in males and reduced gestational age within the range of term gestation are associated with increases in cardiac sympathetic activity. Effects of preterm birth on autonomic indices are not explained by antenatal corticosteroid exposure. Cardiac autonomic imbalance may underlie some of the association between size at birth and cardiovascular risk.

PI-099

Associations of LINE-1 ("Global") DNA Methylation with Preterm Birth in a Prospective Cohort Study. Heather H. Burris[1], Sheryl L. Rifas-Shiman[2], Andrea Baccarelli[3], Caroline E. Boeke[2], Ken Kleinman[2], Xiaozhong Wen[2], Augusto A. Litonjua[4], Janet W. Rich-Edwards[4,5], Matthew W. Gillman[2,6]. *[1]Neonatology, Beth Israel Deaconess Medical Center, Boston, USA; [2]Obesity Prevention Program, Department of Population Medicine, Harvard Pilgrim Health Care Institute and Harvard Medical School, USA; [3]Environmental Health, Harvard School of Public Health (HSPH), USA; [4]Brigham & Women's Hospital, USA; [5]Epidemiology, HSPH, USA; [6]Nutritional Epidemiology, HSPH, USA.*

Little is known about epigenetics and preterm birth. We examined associations of LINE-1 ("global") DNA methylation during pregnancy and at delivery with length of gestation and odds of preterm birth.

Among participants in Project Viva, in white cells from maternal blood during 1st trimester (n=914) and 2nd trimester (n=922), and from venous cord blood at delivery (n=557), we measured long interspersed nuclear element-1 by Pryosequencing (LINE-1, expressed as %5 methyl cytosines [%5mC]). We estimated gestational age at birth from reported last menstrual period or ultrasound. We ran linear regression models to analyze differences in gestation length, and logistic models for odds of preterm birth (<37 v. >=37 weeks gestation), across quartiles of LINE-1.

Mean(SD) maternal age was 32.3(5.1), and 26.4% of women were non-white race/ethnicity. LINE-1 levels were 84.3(0.6), 84.5(0.4), and 84.6(0.7) %5mC for 1st trimester, 2nd trimester and cord blood, respectively. Mean(SD) gestational age was 39.5(1.8) weeks, and 6.5% of infants were born preterm. After adjustment for maternal age, race/ethnicity, BMI, education, smoking status, and fetal sex, women with the highest (v. lowest) quartile of 1st trimester LINE-1 had longer gestation (0.45 weeks [95% CI 0.12, 0.78]) and decreased odds of preterm birth (OR 0.4 [0.2, 0.9]), whereas associations with cord blood LINE-1 were in the opposite direction (-0.48 weeks [-0.87, -0.08] and OR 4.7 [1.2, 18.7]). In models predicting preterm birth, we did not detect effect modification by fetal sex (interaction p-value 0.77 for 1st trimester and 0.56 for cord blood), nor did we detect associations of 2nd trimester LINE-1 with gestational age (0.02 weeks [-0.30, 0.34]) or preterm birth (OR 1.16 [0.56, 2.40]).

Higher 1st trimester LINE-1 DNA methylation was associated with longer gestation and decreased odds of preterm birth. In contrast, shorter gestation was associated with higher LINE-1 in cord blood.

PI-100

Early Neonatal Weight Loss Differs by Mode of Delivery in Healthy Term and Near-Term Neonates. Richard M. Burwick, Thomas D. Shipp. *Obstetrics and Gynecology, Division of Maternal Fetal Medicine, Brigham and Women's Hospital, Harvard Medical School, MA, USA.*

To determine if early neonatal weight loss is influenced by mode of delivery in healthy neonates born after 36 weeks gestation.

Through an electronic database we generated a random sampling of 71 white and black women with non-anomalous, singleton gestations, who received prenatal care and delivered at Brigham and Women's Hospital between 1/1/09 to 1/1/10. Neonates were excluded if they were admitted to the neonatal intensive care unit, delivered prior to 36 weeks gestation, or if they required greater than 3-day stay after vaginal delivery or greater than 5-day stay after cesarean section. We compared neonatal weight loss by mode of delivery categories including vaginal delivery, cesarean section with preceding trial of labor, and cesarean section without labor. In multivariate models of neonatal weight loss we adjusted for parity, race and neonatal feeding method (exclusive breast vs. any bottle).

We abstracted data from 71 charts of subjects meeting inclusion criteria. Mean (±SD) maternal age was 30.8±6.5yrs, with gestational age at delivery of 39.1±1.15wks and birthweight 3194±435g. At hospital discharge, 41.4% of women were exclusively breastfeeding. Mean neonatal weight loss (percent birthweight/day) was significantly greater after vaginal delivery compared to cesarean section (2.06±1.1%/d vs. 1.42±0.88%/d, p=0.008). Furthermore, neonatal weight loss was greater in women who had a trial of labor prior to cesarean section compared to those with cesarean section and no labor (1.78±0.84%/d vs. 0.88±0.69%/d, p=0.003). We found that neonates were significantly more likely to lose greater than 1.5% of their birthweight per day if they had a cesarean section preceded by labor compared to no labor (70.0% vs. 30.8%, p=0.03). In multivariate models, this association remained significant even after adjustment for parity, race, and neonatal feeding method (p=0.042).

Among healthy term and near-term neonates, mode of delivery significantly influences early neonatal weight loss, even after adjustment for confounders. Neonates born after cesarean section without preceding labor experience the least neonatal weight loss. Further research is warranted to determine if any long-term health benefits or consequences exist.

PI-101

Maternal Body Habitus during Pregnancy Is Not a Predictor of Transaminitis in Preterm Infants. Cindy N. Chin[1], Thuan Nguyen[2], Daniel L. Marks[1]. *[1]Pediatric Endocrinology, Oregon Health and Science University, OR, USA; [2]Public Health and Preventive Medicine, Oregon Health and Science University, OR, USA.*

To evaluate maternal body habitus as a predictor of elevated liver enzymes in human infants.

This retrospective review involved de-identified mother-baby pairs from the Oregon Health and Science University (OHSU) electronic medical database. Initial selection criteria included: preterm infant (< 37 wks gestation), available infant alanine aminotransferase (ALT) or aspartate aminotransferase (AST) result, and paired maternal medical record. Maternal body habitus was defined as 1st trimester BMI, gestational weight gain (GWG), and last available pregnancy BMI. Maximum ALT, AST, AST:ALT,

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and triglyceride (TG) values were identified for each infant and used in correlation analyses. The dataset was also analyzed only for those babies who received intralipids (IL) and for those born < 30 weeks.
256 mothers and 300 babies were identified, including 40 sets of twins and two sets of triplets. At delivery, mean maternal age was 29.34 years (± 6.4 years) while average infant gestational age was 32.8 weeks (± 2.8 wks). Of infants born < 30 weeks gestation (n=36), a significant correlation was found between last available pregnancy BMI and infant maximum AST level (r = 0.3585, p-value = 0.03). No other statistically significant correlations were found between maternal body habitus and infant liver labs, including subgroup analyses.
That infant liver enzymes do not generally correlate with maternal body habitus has implications on clinical decisions. Total parenteral nutrition (TPN) in preterm infants is often limited by elevated ALT and AST levels (i.e. transaminitis) as well as TG levels. The current study suggests that a baby's tolerance of TPN, including IL, cannot be predicted based on maternal nutritional markers. The relationship between late pregnancy BMI and infant AST should be re-evaluated prospectively. These results do not exclude the possibility of neonatal NAFLD because elevations in transaminases may not be sensitive markers of NAFLD.

PI-102

Early Phase Growth Evaluation and Prediction by Body Weight Z Score in Relative Long Hospital Stay Preterm Infants. Xi-fang Ru, Qi Feng, Ying Wang, Xin Zhang, Xing Li, Jing-wen Meng, Zai-chen Guo. *Pediatrics, Peking University First Hospital, Beijing, China.*
To illustrate the early phase growth pattern of relative long hospital stay preterm infants, and to explore its influencing and predicting factors.
Enrolled criteria were preterm infant, singleton, admitted in first 24 hours of life, hospital stay longer than 28 days, and clinical followed-up persisted not less than corrected age (CA) 91d (3mo). Body weight Z score and underweight (body weight Z score <-2) incidence were revealed periodically before and at CA 183d. Influencing factors and possible predictors of growth were analyzed. Preterm infants were categorized into three groups by GA (<30wk, 30-31wk, and ≥32wk) or birth weight (<1250g, 1250-1499g, and ≥1500g).
Ninety-one infants (48 boys and 43 girls) were involved. Their GA was 30.9±1.9 wks, birth weight was 1392±312 g, and birth body weight Z score was -1.08±0.77. The lower the birth weight, the lower birth body weight Z score was. Body weight Z scores of all GA and birth weight groups kept on dampening, and the nadir showed up on corrected gestational age (CGA) 36wk. The decline of body weight Z score was quicker and worse in first 3wk in larger GA infants, while long lasting in smaller GA ones. At CGA 40wk, body weight Z scores in all groups returned to birth level. Paralleled with the dynamic shift of body weight Z score, the incidence of underweight reached the peak at CGA 36wk, and then kept at same level of birth. Body weight Z score at CGA 36wk related positively to birth weight, total enteral feeding duration, and gaining weight period, negatively to invasive ventilation duration. CA 61d body weight Z score = -0.300×GA (wk) + 0.210×birth weight (g) + 0.682×body weight Z score at CGA 40wk. Body weight Z score at CA 61d had positive linear regression relationship with body weight Z scores at CA 122d and 183d. The cut-off values of body weight Z score at birth and CA 61d to forecast the risk of underweight at CA 183d were -1.79 and -1.95 respectively.
Early phase growth restriction is a practical problem in relative long hospital stay preterm infants. Smaller GA, lower birth weight and serious early complications are risk factors of early growth. Further dampened growth after birth can be compensated at corrected full term. Body weight Z scores both at CGA 40wk and CA 61d can predict body weight gain before CA 6mo. Poor early life catch up growth happens in lower birth weight Z score babies.

PI-103

A Burst of Organ Development after a Short Postnatal Leptin Supplementation in IUGR Piglets. Linda Attig[1], Thibault Larcher[2], Afif Abdelnour[4], Alison Mostyn[3], Paul Guillauteau[5], Monia Abdennebi-Boukthir[6], Claude Narcisse-Niamba[4], Arieh Gertler[7], Jean Djiane[1], Latifa Abdennebi-Najar[4]. *[1]BDR, INRA, Jouy en Josas, France; [2]UMR 703., INRA, Nantes, France; [3]School of Veterinary Medecine and Science, United Kingdom; [4]EGEAL, Institut Polytechnique laSalle, Beauvais, France; [5]INRA, Rennes, France; [6]Unité de Recherche 04UR08/03, University of Medecine, Tunis, Tunisia; [7]The Hebrew University of Jerusalem, Rehovot, Israel.*
Babies born with intra-uterine growth retardation (IUGR) present an increased susceptibility to develop metabolic diseases in adulthood. We have previously demonstrated in piglets that postnatal leptin supply of IUGR animals could correct their susceptibility to develop metabolic disease. This study aims to investigate the effect of postnatal leptin on thermoregulation, organ development and endocrine profile of IUGR piglets during the development.
At birth, IUGR animals received during a six day either leptin (0.5mg/kg/d, IUGRLep) or saline(IUGRSal) and were compared to their normal birth weight littermates (Control). Colonic temperature were recorded. Plasma triglycerides (TG) and cholesterol were measured on d6. Thyroxine (T4) and cortisol were measured at d21. Some organs were sampled for histological, biochemistry or western blot analysis.
Leptin increased (P<0.05) the relative weight of some organs i.e the liver, pancreas and the small intestine and prevented IUGRLep piglets from the postnatal hypothermia. Leptin normalizes the circulating TG levels on d6, increased plasma T4 and reduced cortisol levels at d21. Leptin induced an increase of the brown adipose tissue content (BAT) and reduced the number of white adipose tissue cells. A normalization of UCP1 expression to control levels was observed in IUGRLep animals. Leptin enhanced the rate of apoptosis in the pancreas and increased the activity of some digestive pancreatic enzymes. Histological changes of the spleen and Peyer plaques were observed in IUGRSal animals arguing for an increased maturation state of these organs of immunity.
All these results suggest that postnatal leptin treatment could alleviate the general developmental delay observed in IUGR. They open interesting therapeutic perspectives in physiopathology to correct defects observed in IUGR.

PI-104

Statins Prevent Adverse Effects of Postnatal Dexamethasone Therapy on Cardiovascular Function in Weanling Rats. K. L. Brain[1], Y. Niu[1], E. A. Herrera[1], E. J. Camm[1], D. Tijsseling[2], J. B. Derks[2], D. A. Giussani[1]. *[1]Physiology Development & Neuroscience, University of Cambridge, United Kingdom; [2]University Medical Centre, Utrecht, Netherlands.*
Dexamethasone (Dex) therapy in premature infants reduces chronic lung disease (CLD) but also triggers cardiac (Bal *et al.*. Ped Res **58**:46,2005) and vascular (Herrera *et al.*. PLoS One, **5**:e9250, 2010) dysfunction in later life. The mechanisms underlying these detrimental effects of Dex are unclear, but oxidative stress with subsequent depletion of nitric oxide (NO) may be involved (Iuchi *et al.*.Circ Res, **92**: 81,2003). In addition to lowering cholesterol, statins increase NO bioavailability (Kaesemeyer *et al.* JACC **33**:234, 1999). Using an integrative approach *in vivo* and at the levels of the isolated heart and vasculature, this study tested the novel hypothesis that combined treatment with statins prevents Dex-induced cardiovascular dysfunction.
Wistar rat pups (52 litters) received a 3-day course of Dex (0.5, 0.3 and 0.1 $\mu g.g^{-1}.day^{-1}$ i.p.) or saline (10 $\mu l.g^{-1}.day^{-1}$ i.p.) ± pravastatin (10 $mg.kg^{-1}$ i.p.) from P1-P3. Pravastatin or vehicle continued from P4-6. One male per litter was used for anyone variable outcome. At P21, *in vivo,* under urethane anaesthesia, cardiac baroreflex responses were generated (phenylephrine, 5-80 $\mu g.kg^{-1}$ i.v.). Following euthanasia, cardiac function was investigated in a Langendorff preparation. Femoral artery reactivity was assessed by wire myography (phenylephrine 10^{-9}M-10^{-5}M and metacholine 10^{-9}M-10^{-4}M).
Relative to controls, Dex increased mean arterial blood pressure (67±2 *vs*. 60±2 mmHg, n=7, *P*<0.05) and baroreflex gain (2.1±0.2 *vs*. 1.3±0.1 bpm. $mmHg^{-1}$, n=7, *P*<0.05). *In vitro,* Dex reduced left ventricular developed pressure (LVDP, 41±3 *vs*. 71±5 mmHg, n=10, *P*<0.05) and myocardial contractility (dP/dt_{max}, 3208.7±250.4 *vs.* 4931.9±324.1 $mmHg.s^{-1}$, n=10,

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$P<0.05$) and delayed cardiac recovery to 15 min of ischaemia. Myography revealed that Dex increased reactivity to phenylephrine but it decreased endothelial dependent relaxation to metacholine. Concomitant pravastatin restored all cardiovascular dysfunction triggered by postnatal Dex. Pravastatin alone had no effects on cardiovascular function.

Statins protect against hypertension, cardiac and endothelial dysfunction following postnatal Dex. Combined glucocorticoid and statin therapy may be safer than glucocorticoids alone in the treatment of CLD in premature infants.

The British Heart Foundation, The BBSRC and Fonds Internationalisering, Utrecht.

PI-105

Fishmeal Supplementation during Gestation Alters the Cortisol Responsiveness of the Offspring Following Maternal Endotoxin Challenge. Rebecca E. Fisher, Herman J. Boermans, Brian W. McBride, Niel A Karrow. *University of Guelph, ON, Canada.*

There has been a lot of interest surrounding maternal consumption of omega-3 polyunsaturated fatty acids (n-3 PUFAs) and its beneficial affects on the programming of the fetus. Positive correlations between maternal n-3 PUFA supplementation and a decreased risk of allergy, asthma and psychological disorders in the offspring have been observed. Therefore, the objective of this study was to examine the responsiveness of the HPA axis of offspring born to mothers that had been supplemented with fishmeal (FM) or soybean meal (SM).

Forty-four ewes were allocated to either a diet supplemented with FM (n=23) or SM (n=21). On day 135 of gestation half the ewes from each dietary treatment group were challenged with either 1.2 µ/kg Escherichia coli endotoxin (ENDO) administered i.v. or saline (CON) as control. At weaning offspring were challenged with 0.5 µ/kg ACTH to assess their coritsol response. Blood samples were taken at T0, 6, 24 hours post weaning with ACTH being administered at 24 hours post weaning and additional blood samples taken at 0, 0.25, 0.5, 1, and 2 hours post injection. At 5.5 months of age offspring from all treatment groups were challenged with 400 ng/kg of ENDO with blood samples drawn at 0, 2, 4 and 6 hours post challenge.

All offspring responded to both the ACTH and ENDO challenge with changes in cortisol concentration over time. Following the weaning and ACTH challenge it was observed that offspring born to mothers supplemented with either FM or SM and challenged with CON had an increased cortisol response at 24 hours post weaning compared to the offspring born to mothers supplemented with FM or SM and challenged with ENDO. Interestingly however, offspring born to mothers challenged with ENDO and supplemented with FM had an increased cortisol response compared to other treatment groups at 0.5 hours following the ACTH challenge. This was also observed following the offspring ENDO challenge at 5.5 months of age with an increased cortisol response in female offspring born to mothers supplemented with FM and challenged with ENDO compared to the other treatment groups. Additionally the cortisol response also peaked earlier in these offspring compared to the other treatment groups.

Results from this study suggest that FM supplementation during gestation coupled with ENDO challenge leads to alterations in the stress responsiveness of the offspring on into adulthood.

PI-106

Early Endothelium-Dependent Vasodilatory Dysfunction in Normotensive, Intra-Uterine Growth Restricted Rats Is Restored by L-Arginine and Inhibition of Arginases. Isabelle Grandvuillemin[1,2], Christophe Buffat[1,2], Farid Boubred[1,2], Isabelle Ligi[1,2], Philippe Charpiot[2], Françoise Dignat-George[2], Umberto Simeoni[1,2]. *[1]Université de la Méditerranée, INSERM UMR 608, Marseille, France; [2]Division of Neonatology, Hôpital de la Conception, Assistance Publique - Hôpitaux de Marseille, Marseille, France.*

Increasing clinical and experimental evidence demonstrates that fetal programmed hypertension in adulthood is associated with altered endothelium-dependent vasodilation. However, it is debated whether decreased endothelium-dependent vasodilation precedes or is a consequence of elevated arterial pressure. We studied endothelium-dependent vasodilating capacity and the L-arginine-nitric oxide pathway in intra-uterine growth-restricted (IUGR), 5-week-old, male rats, before the development of elevated arterial pressure. We furthermore tested the hypothesis that alteration in endothelial vasodilation may be due to a reduction in L-arginine bioavailability.

Pregnant rats were fed either a control or protein restricted diet during gestation. Endothelium-intact aortic rings of 5-week-old male offspring of the control and UGR groups were isolated and preconstricted with phenylephrine (PE) to test vascular reactivity to acetylcholine (Ach) and sodium nitroprusside (SNP), respectively endothelium-dependent and endothelium-independent vasodilators. Other experiments were conducted with L-arginine supplementation or with BEC, a specific endothelial arginase inhibitor. Arginase enzymatic activity was measured in aorta.

Systolic blood pressure was not different between groups, and increased later in IUGR rats.While the vasodilatory response to SNP was similar in the two groups, a decreased vasodilatory response to Ach (- 20%, $p<0.05$) was observed in the IUGR group. Moreover, L-arginine supplementation and arginases inhibition restored the altered vasodilating capacity of aortic rings of IUGR animals.

Reduced endothelium-dependent vascular relaxation precedes the development of fetal programmed hypertension in IUGR rats and involves the L-arginine-NO pathway. L-arginine supplementation and inhibition of arginases restore endothelium-dependent vasodilation in IUGR, pre-hypertensive rats. Arginase activity is enhanced in aorta of young IUGR rats.

PI-107

A Measurement Model of Infants Physical Activity Using Pedometer. Joao G. Alves[1], Felipe Sarinho[1], Vivianne Barros[2], Melania M. Amorim[1]. *[1]Pediatrics, Instituto de Medicina Integral Prof Fernando Figueira (IMIP), Pernambuco, Brazil; [2]Pediatrics, UFPB, Paraiba, Brazil.*

Physical activity tracks from childhood to adulthood. However the physical activity patterns in infancy is unknown. Our aim is to assess the level of pedometer-physical activity in infants and its correlation with weight gain.

This is a cohort study with 60 healthy newborns followed during the first trimester of life. Physical activity was measured using a pedometer applied in the feet of the infants to measure their leg movements. Pedometer-Physical activity, cycles of leg movements determined by pedometer reading, was compared between the first and the third month of age in each child. All the mothers were taught how to use the pedometer in their infants. The pedometer (Yamax Digiwalker SW-200) was applied in the right foot, fixed through the footwear of the child. Pedometer was used during four consecutive days being reseted every morning and it was not used during sleep and bath times. The average of the four readings was used to do the analysis. Weight and height were measured.

Newborns showed 440 to 720 (580 + 77) cycles of leg movements per day. At third month of age these infants increase the number of cycles to 1,027 + 103; 910 to 1,113. It was observed a negative correlation between pedometer readings (cycles of leg movements) and weight gain.

The level of physical activity in infants increases during the first months of life. Overweight infants are less physically actives.

PI-108

Opportunities for Public Dissemination of DOHaD Concepts. Stephen A. Bezruchka. *Global Health, University of Washington, WA, USA.*

Lay awareness of the importance of early life for adult health lags behind far research findings and theory. Perceptions vary among various cultures with western thought and individual agency hampered by myths such as "we are all created equal." Eastern perspectives may be more receptive.

Academic researchers focus on specific short-term questions and are hesitant to generalize findings. Media attention is rarely paid to the intangible concepts underlying DOHaD. The medical care industry lacks an laboratory test, therapeutic procedure or pharmaceutical intervention related to early life.

What can be done to create awareness of DOHAD?

Specific avenues for creating awareness depend on the target audience. There are possibilities for enhancing grade school curriculums and beyond to highlight that fetal and child circumstances affect adult health. Requiring proficiency in describing early life issues related to health in

adulthood could be incorporated into standardized exams such as the SAT and O-levels. Certifications for various types of health care workers can require demonstrating understanding of DOHaD issues.
Research can be enhanced by having vital statistic records throughout the lifespan with recording of parameters such as gestational age estimates, birth weights, physical and social environmental attributes, socioeconomic parameters, and parental attachment issues before school entrance. Clinical record keeping can have similar entry fields and a standardized format globally could allow for longitudinal data tracking.
Today there are various IT and entertainment opportunities to create videos, quizzes, games, movie stories, songs and raps as well as traditional print media to present these concepts.
Legislation can require attention paid to inadequate early life as qualifying for disability status in the same way as does lack of vision, a spinal cord injury or a congenital malformation.
Examples of teaching methods at the high school or college level presenting DOHaD concepts will be reviewed.
Creating awareness that early life impacts a lifetime presents important opportunities for those researching DOHaD.

PI-109

Familial Income Predicts Neuroanatomical Correlates of Depression in Infancy. Anne Rifkin-Graboi[1], Siti Aishah Bte Abdul Rahman[1], Lit Wee Sim[2], Muhammed Farid Abdul-Rahman[2], Marielle V. Fortier[3], Helen Chen[3], Cornelia Chee[4], Kenneth Kwek[3], Yap Seng Chong[4], Seang Mei Saw[4], Keith Godfrey[5], Peter D. Gluckman[1,6], Michael J. Meaney[1,7], Anqi Qiu[2]. *[1]Singapore Institute of Clinical Sciences, Singapore; [2]National University Singapore, Singapore; [3]KK Hospital, Singapore; [4]National University Hospital, Singapore; [5]University of Southampton, United Kingdom; [6]University of Auckland, New Zealand; [7]McGill University, Canada.*
Mood disorders are greater amongst children born into poverty—a condition often comprising familial violence, poor nutrition, and chronic stress. However, the mechanisms contributing to this association remain unclear. For example, do socio-economic factors operate during prenatal development to influence biological endophenotypes of depression? Does parental socio-economic adversity contribute to the vulnerability for depression? We examined neonatal amygdala white matter, a biological correlate of depression, as a function of Singaporean familial monthly income.
Participants were members of the "Growing Up in Singapore Towards Healthy Outcomes (GUSTO)" cohort study. Mothers reported monthly income, as well as depression and anxiety levels at 26 weeks gestation. Infants underwent Diffusion Tensor Imaging (DTI) to measure amygdala axonal maturity (Fractional Anisotropy, FA) at 7 – 10 days of life. The median income of families with neonates participating in DTI (roughly 4500 SGD) was similar to Singapore's median income.
To ensure any observed relations were not a bi-product of maternal depression, we examined the relations between maternal mood and a) income and b) separately, infant left and right FA. In only one case was there a trend level association; after controlling for maternal depression and infant age monthly income significantly predicted right amygdala FA (B (3, 60)= .255, p = .03); after controlling for infant age, income significantly predicted left amygdala FA (B (2, 106)= .248, p = .01).
This is the first study demonstrating effects of familial income upon the neonatal amygdala. These results may imply that *relative* economic disadvantage influences brain structures associated with depression. Income effects were not due to maternal mood, and post-natal experience appears an unlikely mechanism considering the age of the infants. Thus, interventions focused upon lower income mothers prior to delivery may be of great benefit in reducing the risk of depression in the offspring.

PI-110

Timing and Secular Trend of Pubertal Development in Chinese Girls. Fangfang Chen[1], Youfa Wang[2], Jie Mi[1]. *[1]Department of Epidemiology, Capital Institute of Pediatrics, Beijing, China; [2]Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, MD, USA.*
To provide the overview of current median age of various pubertal characteristics, and the secular trend in menarcheal age from 1940s to the present among females in Beijing, China.
Six data sets were used, including those from 9,778 girls aged 6-18 years in the Beijing Child and Adolescent Metabolic Syndrome Study (BCAMS Study) in 2004. The status quo method was used to calculate the median age of pubertal characteristics. The FOAD (Fetal Origins of Adult Disease) cohort study provided information of menarcheal age in 1940s and 1960s. Four other studies were examined to obtain supplementary information for the secular trend analysis. Linear regression method was used to evaluate the trend.
In BCAMS, the median age at menarche (MAM) was 12.1 (SD: 1.1) years for the whole sample, and it's 0.6 years earlier in urban than in rural girls. For all, urban and rural girls, median age at breast Tanner stage 2 was 9.5±1.2, 9.4±1.1, and 9.6±1.2 years, respectively. Median age at pubic hair Tanner stage 2 was 11.1±1.1, 10.8±1.1, and 11.4±1.1 years, respectively. In FOAD, MAM was 14.9±1.7 years in the 1940s and 13.4±1.4 years in 1960s. MAM in urban girls was advanced by 7.8 months per decade between 1940s and 1960s. The rate decreased to 4.5 months per decade between 1985 and 2004, while the rate was lower in rural girls of 2.6 months per decade.
Females in Beijing have matured earlier, while the secular trends seem slowed down in recent decades. Urban girls matured earlier than rural girls.

PI-111

Do Parental Reports of Infant Temperament Predict Negative Affect and Self-Regulation? Seok Hui Tan[1], Alice Yeo[1], Erin Fu[1], Jen Richmond[2], Anne Rifkin-Graboi[3], Yap Seng Chong[4], Kenneth Kwek[5], Seang Mei Saw[2], Peter D. Gluckman[3,6], Michael J. Meaney[3,7], Shang Chee Chong[4]. *[1]National University Singapore, Singapore; [2]University of New South Wales, Australia; [3]Singapore Institute of Clinical Sciences, Singapore; [4]National University Hospital, Singapore; [5]KK Hospital, Singapore; [6]University of Auckland, New Zealand; [7]McGill University, Canada.*
Infant temperament is an important predictor of subsequent physical and emotional health, but the degree to which parentally rated infant temperament relates to observed infant behavior is less well understood. External validation of infant temperament measures is essential to understanding the mechanism through which temperament influences health trajectories.
Mother-infant dyads were part of a larger longitudinal study: Growing Up in Singapore towards Healthy Outcomes sampling 1388 healthy fullterm infants. As part of this study, mothers completed the Infant Temperament Questionnaire (ITQ: Carey & Mcdevitt, 1978) when infants were three months. A subsample (n=450) are scheduled to take part in a Deferred Imitation Task, measuring memory for a novel puppet, at six months. Videos ofthis task were scored for emotional reactivity (fear of the puppet, anger at initial restraint, and withdrawal), and self-regulation in the form of attention to the experimenter rather than puppet. At the time of abstract submission, ITQ data were available for 97 infants and infant behaviours for 21 dyads (11 were primaparous mothers; none had a mental illness diagnosis).
Preliminary results indicate that temperament ratings correlated with fear (but not anger) expression and withdrawal at the first puppet presentation. Infants rated low for Activity showed more fear, r(20)=-.68, p=.001, and withdrawal, r(20)=-.69, p<.001. At the second puppet presentation, infants with high Mood and low Distractability were more likely to attend to the experimenter, r(20)=.47, -.44 respectively, ps<.03; infants with high Mood ratings also attended less to the source of their distress (puppet), r(20)=-.45, p=.04. Relationships between withdrawal and Threshold and Adaptability ratings, approached significance, rs(20)=-.39, ps=.08. Less irritable infants showed more fear, while expressive and less distractable infants showed more self-regulation three months later.
Infant temperament reported using the ITQ can be useful in identifying infants at risk for childhood health difficulties associated with difficult temperament.

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PI-112

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

An adverse *in utero* environment has been shown to program the stress axis in later life. Previous studies have shown inconsistent associations between birth weight and basal HPA activity possibly due to limited sample sizes, cohort heterogeneity and the potential "stress responsiveness" of collecting samples in a novel clinic setting. *The Aim of this study was to investigate the association between birth measures and basal HPA activity in late adolescence.*

The Western Australian Pregnancy (Raine) Cohort recruited 2900 pregnancies at 18 weeks gestation and 2868 offspring have undergone detailed phenotyping. Basal HPA activity was assessed at 17-years. Awakening salivary samples were collected on three successive mornings for cortisol determination (salCORT). On the third morning a fasting blood sample was collected and the plasma analysed for cortisol (totalCORT), ACTH and CBG. Unbound cortisol (freeCORT) in plasma was calculated using Coolen's equation. All samples were collected in a home environment. Multivariate regression analysis was used to investigate associations between birth measures (weight, length, abdominal circumference, ponderal index) and basal adolescent HPA activity. Males (n=707) and females (n=693) were analyzed separately.

In females there were no associations between birth measures and basal HPA activity. In males, associations were identified between measurements at birth and salCORT but not for totalCORT, freeCORT or ACTH. salCORT was positively associated with birth weight (p=0.024; range 915g-5550g), birth length (p=0.008) and abdominal circumference (p=0.023). No associations were observed with ponderal index or weight gain during first year of life. The associations in males were no longer significant if preterm babies (n=49) were excluded from the analyses.

In babies born at term there is no association between measurements at birth and basal HPA activity in late adolescence. The association between lower birth weight and awakening salivary CORT appears to be limited to preterm males. These observations require replication in other studies where HPA function is measured under basal conditions.

PI-113

Vulnerability to a Nutritional Deficiency of N-3 Polyunsaturated Fatty Acids (N-3 Pufas) by Exposure to Early Stress – Effects on Behavior and Metabolism. Juliana R. Bernardi[1], Charles F. Ferreira[1,3], Gabrielle Senter[1], Ana Paula S. Huffel[3], Rachel Krolow[3], Danusa M. Arcego[3], André K. Portella[1], Márcia K. Sant'anna[2], Marcelo Z. Goldani[1], Carla Dalmaz[1,3], Flávio P. Kapczinski[2], Patrícia P. Silveira[1]. *[1]Núcleo de Estudos da Saúde da Criança e do Adolescente - Dpto Pediatria, Hospital de Clínicas de Porto Alegre - Universidade Federal do Rio Grande do Sul, Brazil; [2]Depto. Psiquiatria, Universidade Federal do Rio Grande do Sul, Brazil; [3]PPG Neurociências - Depto Bioquímica, Universidade Federal do Rio Grande do Sul, Brazil.*

Interventions in early life are associated with persistent alterations in metabolism, behavior and susceptibility for diseases in adulthood. We aimed at studying the interaction between early stress and chronic nutritional deficiency of n-3 PUFAs (polyunsaturated fatty acids) on behavioral and metabolic outcomes.

Male rats were randomized into non-handled (NH) or maternal separation (S) (incubator at 34°C, 3 hour/day from 1st to 10th postnatal days). At day 35º of life, rats were randomized into diet adequate or deficient in n-3 PUFAs for 15 weeks. We measured animals' body weight, food consumption, abdominal fat, plasma/hepatic triglycerides (TG) and parameters related to oxidative stress in heart tissue. Statistical tests used were two-way or repeated ANOVA. Significance levels were set at $p<0.05$.

There was a group and a diet effect, where S had higher weight (p=0.027) and food consumption (p=0.022) than NH, and the n-3 deficient diet decreased the body weight of all groups (p=0,01), without interactions. In relation to abdominal adiposity and plasma TG, there was a neonatal intervention effect, in which S showed heavier abdominal fat depots ($p<0.001$) and more plasma TG (p=0.018), without interaction with the diet. However, the n-3 deficient diet seems to decrease the hepatic TG (p=0,061). Regarding cardiac oxidative stress, superoxide dismutase (SOD, antioxidant) showed a group effect (p=0.048) where S had lower activity than NH, without diet effect or interactions. DCF (a marker of cellular oxidative stress) was increased in S (p=0.05) and there was a borderline interaction with diet, increasing DCF in S/n-3 deficient rats (0.07).

These data suggest that the neonatal environment can alter the metabolism in response to an early stress and a diet deficient in n-3 PUFAs. This model can be a useful tool for studying the interaction between the early environment and the life-course nutrition on different outcomes.

PI-114

Anxious Pregnant Woman Less Able To Adapt to Stressful Situations. Marijke A.K.A. Braeken, Renée A. Otte, Bea R.H.M. Van den Bergh. *Developmental Psychology, Tilburg University, Netherlands.*

The Autonomic Nervous System (ANS) helps women to adapt to all physiological and psychological changes they experience during pregnancy. Maladaptation to these changes may have negative effects on the child's cognitive, emotional and behavioral development. The interaction between the two subsystems of the ANS (sympathetic and parasympathetic pathway) is expressed by heart rate variability (HRV) and a malfunctioning ANS can be detected by a lower HRV. We investigated the relationship between prenatal anxiety and HRV to assess their potential influence on the offspring's development.

During the third trimester of pregnancy (week 31-37 of gestation) 107 women filled out the Anxiety scale of the Symptom Checklist. Additionally, the women's HRV was recorded, continuously for 25 minutes, while they completed a task. The task consisted of watching relaxing pictures and listening to calming music (3x5') alternated twice with an arithmetic task (2x5').

The SCL's anxiety scale had significant effects on several HRV measures during the stressful phases in the arithmetic task (i.e. phase 2 and 4). SCL was significant associated with HRV triangular index in phase 2 ($p < 0.05$) and 4 ($p < 0.01$), with TINN in phase 2 ($p < 0.05$) and 4 ($p < 0.01$) and with HF in phase 2 ($p < 0.05$) and 4 ($p < 0.05$). Additionally, SCL was also found to be significant related to SDNN in phase 4 ($p < 0.05$) and LF in phase 4 ($p < 0.05$).

We can conclude that women at the end of pregnancy who are more anxious have a lower HRV in an acute stressful environment. The results of this study indicate that pregnant women who experience anxious feelings are less able to cope adequately with stressful situations. This potentially may lead to less favourable outcomes for the child.

PI-115

Exposure to Different Types of Diets during Gestation and Lactation Period Influence Maternal Care, Milk Composition and Body Weight of Neonatally Handled and Non-Handled Pups. Carla da Silva Benetti, Hellena Gonçalves Vido, Roberta Dalle Molle, André Krumel Portella, Fernanda Urruth Fontella, Isabel Werlang, Juliana Bernardi, Mariana Dihl Schiffner, Carla Dalmaz, Patrícia Pelufo Silveira, Marcelo Zubaran Goldani. *Universidade Federal do Rio Grande do Sul-UFRGS, Brazil.*

Background: Early environment determines neuroendocrine and metabolic alterations that persist to adulthood. Exposure to malnutrition and obesity-induced diet during critic periods of development, such as gestation and lactation, is associated to adiposity and altered glucose, insulin as well as leptin metabolism in offsprings. Experimental models demonstrate that post-natal environment affects maternal care and metabolic response to chronic palatable diet in adult neonatally handled rats. Aims: Verify if the exposure to different types of diets during gestation and lactation period would alter the maternal care, milk composition, milk leptin concentration and body weight of neonatally handled and non-handled pups.

Methods: On the day one of gestation, the dams were randomized to receive: (C) control, (LP) low protein (8%) or (HF) high fat diet (45%). Consumption and body weight were accompanied during gestation and the first six days of lactation. After birth, maternal care was observed (5 sections, 72 min. each)

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from post-natal day (PND) one to six. Pups were handled or not (maternal separation, 10 min/day) during the first 6 PND. On the day 6 of lactation, pups were weighed and the dam's milk was collected.
Results: HF diet seems to increase maternal care, while the neonatal handling seems to revert a reduction in maternal care induced by the LP diet. Increased carbohydrate concentration was found in milk of dams exposed to HF diet whose pups were neonatally handled. The exposure to LP diet during gestation reduced protein in milk; however, neonatal handling reverted this effect, increasing protein amount. Although milk fat composition was not affected by the diet, increased leptin content in milk was induced by the LP diet. Pups whose dams were exposed to LP diet exhibited decreased body weight on the PND 6.
Conclusions: Interventions during critic periods modify the maternal care, milk composition, as well as milk leptin concentration. It may be suggested an interaction between exposure to different sorts of diets during the perinatal period, and an influence of neonatal handling on these parameters.

PI-116

Brain-Derived Neurotrophic Factor Levels and Behavioral and Metabolic Responses Later in Life in an Animal Model of Early Life Stress. Roberta Dalle Molle[1], André K. Portella[1], Fernanda U. Fontella[1], Carla S. Benetti[1], Adolfo R. Reis[1], Giovanni A. Salum[2], Gisele G. Manfro[2], Marcelo Z. Goldani[1], Patrícia P. Silveira[1]. *[1]Núcleo de Estudos da Saúde da Criança e do Adolescente - Depto. Pediatria, Hospital de Clínicas de Porto Alegre – Universidade Federal do Rio Grande do Sul, Porto Alegre/RS, Brazil; [2]Departamento de Psiquiatria, Hospital de Clínicas de Porto Alegre, Porto Alegre/RS, Brazil.*
Use an animal model of early life stress to assess body weight gain, food intake, anxiety-like behavior and peripheral and central levels of brain-derived neurotrophic factor (BDNF) later in life.
By the second day of life, ten litters of Wistar rats and their dams where divided in two groups: intervention, with limited access to nesting material, or control. Maternal behavior was observed during the seven days of intervention. After weaning, animals' weight and standard chow consumption were measured once a week. Starting on day 60 of life, rats were submitted to a test that evaluates the preference for palatable food (sweet) and to the elevated plus maze test that assessed anxiety-like behavior. After decapitation, plasma and hippocampus, amygdala and periaqueductal gray BDNF contents were measured.
Intervention dams showed less variation on the total duration of licking and grooming than the control dams. No difference in body weight gain was observed between groups. Nevertheless, the intervention group consumed less standard chow than the control group ($p=0,008$), but intervention females consumed more sweet food ($p<0,001$). On the plus maze test there was an interaction between group and sex ($p=0,02$), in which the intervention males spent less time in the open arm, indicating a higher anxiety-like behavior; both males and females of the intervention group demonstrated decreased frequencies of head dips ($p=0,007$), which is a behavior related to environmental exploration. An effect of group was observed in plasma BDNF levels ($p=0,05$), showing that intervention was associated with higher plasma levels of this neurotrophin. There was no difference in hippocampus, amygdala and periaqueductal gray BDNF contents.
The early life stress, able to alter the relationship between dam and pup, have a persistent impact on food intake, anxiety-like behavior and plasma BDNF levels, being this animal model interesting to study the influence of variations in early life environment on neuroendocrine, metabolic and behavioral responses later in life.

PI-117

Maternal Protein Restriction Leads to Pulmonary Artery Remodelling in Adult F1 Rat and Young F2 Offspring. Christopher Torrens[1], Shelley A. Davis[2], Joel Byfield[1], Fiona Thomas[1], Jaya Kane[1], Graham Burdge[1], John Holloway[2]. *[1]Human Health and Development, University of Southampton, United Kingdom; [2]Infection, Inflammation and Immunity, University of Southampton, United Kingdom.*
Environmental challenges during early life have been shown to result in greater risk of chronic diseases such as diabetes and coronary disease in later life. Factors such as unbalanced nutrition before birth result in metabolic and structural adaptations that lead to persistent modifications to offspring phenotype. There is also evidence that respiratory disease is influenced by developmental environment, where reduced fetal growth is associated with lung development and increased risk of developing COPD. (Barker *et al.* 1991.) The aim was to investigate whether exposure to a low protein diet *in utero* affects adult offspring lung morphology.
Pregnant Wistar rats fed either control (C, 18% casein) or protein restricted (PR, 9% casein) diet from conception to term, and were then returned to standard chow. Lung tissue was harvested (28 days and 225 days F1) from male offspring, female offspring were used to create F2 generation from which lungs were harvested at 28 days. Whole left lungs were fixed in formalin, embedded in paraffin wax and sectioned. Sections were H and E stained, and morphological measurements were made give volume fractionation and to estimate surface area; at 10x magnification, and alveolar wall thickness; 63x magnification. Volume was calculated by volume displacement.
Significant increase ($p=0.046$, T-test) in the amount of smooth muscle around the vessels in the protein restricted group compared to the control group for both the 225 day F1 and 28 day F2 time points. No other differences were seen.
The increased smooth muscle data suggests remodelling in the pulmonary arteries and may be indicative of pulmonary hypertension in this model. In this study there is no evidence to suggest that *in utero* exposure to a maternal low protein diet has significantly affected adult lung weight or volume these rats.

PI-118

Influence of Maternal Birth Weight on Offspring Birth Weight in the Western Region of São Paulo: The Butantã Cohort. Ana Maria U. Escobar[1], Maria Helena Valente[1], Filumena Maria S. Gomes[1], Luis Marcelo I. Cirino[2], Leide Irislayne M. Araujo[1], Isac de Castro[1], Alexandra Brentani[1], Sandra Josefina F.E. Grisi[1]. *[1]Pediatrics, Faculdade de Medicina da Universidade de São Paulo, SP, Brazil; [2]Surgery, Faculdade de Medicina da Universidade de São Paulo, SP, Brazil.*
Introduction: Insults during embryo development may lead to adverse effects in later life to the both to the individual and his next generations. Studies have shown that maternal malnutrition was associated with greater child weight in the next two generations, even if they had a normal and healthy diet. In developing countries the obesity is an endemic problem, associated with socio-economic transition. We also observe in prenatal examinations a high incidence of intrauterine growth retardation due to structural problems. Such finding becomes very important since it leads to increased risk of nutritional disorders in later generations. **Objective:** To study mother´s birth weight influence on offspring birth weight.
A retrospective cross-sectional study comprising 122 women and their children, followed in Butantã Cohort was conducted. This cohort is located in the western region of São Paulo City and is part of a research project of the Pediatrics Department. Clinical history and review data from pediatric clinical record, including a collection of information on mother´s and children's birth weight were obtained. Statistical analysis was performed by the cumulative frequency of weight sequentially analyzed with Pearson's chi-square test for independent groups and expressed as proportions. Two tailed p values ≤0.05 were considered significant. Odds ratio for risk estimate was calculated.
When analyzing women who were born weighting less than 3.000Kg, 49.3% (n=35) had children born with less than 3.0Kg, compared to 27.5% (n=14) mothers born with at least 3.0Kg , with a significant difference $p=0.0152$, OD 2.6, 95% CI = 1.2-5.6.
We found a significant association between maternal and child birth weights correlated to mothers born weighting less than 3,000Kg who had children, also bornLer foneticamente weighting less than 3,000Kg.

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PI-119

Intergenerational Effects of Maternal Height on Offspring Birth Weight in the Western Region of São Paulo: The Butantã Cohort. Ana Maria U. Escobar[1], Filumena Maria S. Gomes[1], Maria Helena Valente[1], Leide Irislayne A. Macena[1], Luis Marcelo I. Cirino[2], Isac de Castro[1], Alexandra Brentani[1], Sandra Josefina F.H. Grisi[1]. *[1]Pediatrics, Faculdade de Medicina da Universidade de São Paulo, São Paulo/SP, Brazil; [2]Surgery, Faculdade de Medicina da Universidade de São Paulo, São Paulo/SP, Brazil.*

Introduction: It is believed that the intergenerational cycle of "growth failure" where women with short stature are more likely to have offspring with low birth weight, confirms the perpetuation mechanism of programming through generations.

Aims: To evaluate the relationship between maternal height and offspring birth weight.

We performed a longitudinal and retrospective study of 625 mothers of infants enrolled in the Butantã Cohort from January 2007 to December 2009. The Butantã cohort is located in the western region of São Paulo City and is part of a research project of the Pediatrics Department. Maternal height percentile and offspring birth weight were evaluated. Women's measures were classified in height percentiles, according to World Health Organization standards, adjusted for age. Percentiles (P) in height were considered: <P15, between P15 and P50; ≥ P50. Birth weight was stratified as: less than 2,500 g, from 2,500 to 3,000 g, 3,000 to less than 3500 g, between 3500-4000g and 4000 g or more. Mother´s height percentile frequencies were analyzed according to offspring birth weight range in bands for cumulative assessment of risk and better discrimination. Pearson's chi-square test was performed for independent groups and expressed in proportions. The odds ratio for risk estimate was also calculated.

51% of mothers (n=101) classified as height percentile 15 or less had newborns weighing between 2.5 and 3 kg, compared to 33.5% mothers (n=143) in height percentile > 15 with a significant difference ($p < 0.0001$), Odds = 2 (1.5-2.9). We also observed that 2% of mothers (n=4) in height percentile < 15 had children born weighing more than 4.0Kg, compared to 6.6% (n=17) in height percentile >15 < 50 and 10.8% (n=18) mothers in height percentile > 50 with a significant difference between height percentiles, ($p = 0.0005$).

There is a significant association between offspring born weighing 3,000 g or more and maternal height. Women in the P15 height percentile had twice the risk of having children weighing less than 3000 g.

PI-120

Intergenerational and Early Life Determinants of Cardiovascular Risk Factors in Swedish Children. Amal Khanolkar[1,3], Liisa Byberg[2], Ilona Koupil[1]. *[1]Centre for Health Equity Studies (CHESS), Karolinska Institutet/ Stockholm University, Sweden; [2]Uppsala Clinical Research Center and Department of Surgical Sciences, Uppsala University, Sweden; [3]Institute for Environmental Medicine, Karolinska Institutet, Sweden.*

To study the relationship of children's cardiovascular (CVD) risk factors with parent's CVD risk factors (RF), socioeconomic position (SEP) and lifestyle.

602 families (2141 individuals), with two full sibs; aged 5-14 years and their biological parents (Uppsala Family Study) formed the study population. Cholesterol, ApoB/ApoA1 ratio, adiponectin, blood pressure and BMI were measured by routine methods. Age and gender specific overweight/obesity was computed. Parental SEP determined by occupational class and education and lifestyle habits (alcohol consumption, smoking, physical activity) were from questionnaires. Associations with CVD RF were analysed by linear and logistic regression. Results were adjusted for child's age, gender, pubertal stage and family clustering.

Strong, consistently significant associations exist between parents' and children's CVD RF independent of parental SEP and lifestyle. Certain parental lifestyles, adjusted for SEP, had strong significant associations with children's CVD RF. Children of smoking parents had higher BMI (ratio smoking vs. non-smoking fathers and mothers 1.04, 95% CI 1.00-1.17 and 1.03, 1.00-1.15). Children of mothers reporting vigorous physical activity had lower BMI, cholesterol and decreased odds for overweight/obesity in a dose response manner. Compared with mothers reporting no vigorous activity, mothers with <1.1 5 hours and 1.15-2.4 hours/week vigorous activity had 43% (OR 0.57, 95% CI 0.22-0.89) and 72% (0.28, 0.14-0.60) lower risk of having an overweight/obese child respectively. Alcohol consumption in parents was significantly associated with higher cholesterol and BMI (mothers only) in children. We found few independent associations between parental SEP and children's CVD RF.

Strong correlations in CVD RF between family members that are not related to parental SEP/lifestyle suggest a role of genetics in influencing children's CVD RF and early development of these risk factors in childhood. Parental behaviours; smoking, alcohol consumption and low physical activity were significantly associated with higher levels of some CVD risk factors in children. Both maternal and paternal smoking appears equally important. Public health policies should target families with unhealthy lifestyles.

PII-121

Fetal Programming of Infant State Regulation. Gerald F. Giesbrecht[1], Brenda Leung[1], Nicole Letourneau[2], Tavis S. Campbell[1], Bonnie J. Kaplan[1]. *[1]Paediatrics, University of Calgary, AB, Canada; [2]Nursing, University of New Brunswick, NB, Canada.*

The goal of this study was to extend previous research linking antenatal psychological distress with fear reactivity in infants. We prospectively assessed cortisol and self-reported mood in pregnant women in relation to various aspects of state regulation in their infant offspring.

Depressed mood and positive mood (Profile of Mood States), and cortisol were assessed (5 samples/day X 3 days) in 76 pregnant women. Patterns of diurnal cortisol were determined from saliva. At three months postpartum, mothers reported their symptoms of depression (Edinburg Postnatal Depression Scale) and anxiety (Symptom Checklist 90) as well as their infant's sleep consolidation (Brief Infant Sleep Questionnaire), crying (Crying Patterns Questionnaire), and two components of temperament - regulation (of attention and arousal) and negative affect (Infant Behavior Questionnaire – Revised).

We used hierarchical linear regression to determine whether prenatal maternal mood and cortisol predict infant temperament, sleep, and crying after accounting for the effects of postnatal maternal mood. In each model, postnatal anxiety and depression were entered first (step 1) followed by depressed and positive mood and cortisol assessed during pregnancy (step 2). Fetal sex and interactions between sex and maternal predictors were included to test whether sex moderates these associations. Step 2 was significant for each of the models (all Fs > 2.6, all ps < .05), indicating that prenatal mood and cortisol were significant predictors of infant state regulation. For temperament regulation there was a main effect of positive mood and a cortisol by sex interaction. Higher levels of positive mood were associated with better regulation for boys and girls while higher cortisol predicted reduced regulation in boys only. For temperament negativity there was a main effect of depressed mood. Increased depressed mood was associated with greater infant negative reactivity. In addition, a blunted maternal cortisol awakening response was marginally ($p < .1$) associated with increased negativity. For both the crying and sleep models, higher levels of depressed mood were associated with poor outcomes only in boys.

Both psychological and physiological aspects of maternal psychological distress during pregnancy were associated with infant state regulation. Overall, antenatal psychological distress appears to be more disruptive for boys than girls.

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PII-122

Prenatal Growth and the Risk of Suicide: The Helsinki Birth Cohort Study. Marius Lahti[1], Katri Räikkönen[1], Kristian Wahlbeck[2], Anu-Katriina Pesonen[1,3], Kati Heinonen[1], Jari Lahti[1], Eero Kajantie[2,3], Clive Osmond[4], David J.P. Barker[5], Johan G. Eriksson[2,6,7,8,9]. *[1]Institute of Behavioural Sciences, University of Helsinki, Finland; [2]National Institute for Health and Welfare, Finland; [3]Hospital for Children and Adolescents, University of Helsinki, Finland; [4]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom; [5]DOHaD Centre, University of Southampton, United Kingdom; [6]Department of General Practice and Primary Health Care, Helsinki, University of Helsinki, Finland; [7]Vaasa Central Hospital, Finland; [8]Unit of General Practice, Helsinki University Central Hospital, Finland; [9]Folkhälsan Research Center, Finland.*

Findings on the effects of fetal growth on the risk of suicide are ambiguous. Hence, we examined in the Helsinki Birth Cohort Study 1934-44 whether body size at birth and/or length of gestation predicts suicide risk in adulthood.

We extracted data on body size at birth and length of gestation from birth records and on suicide from the Finnish National Causes of Death-register. Of the 5942 women and 6517 men in the current study, 22 women and 92 men had committed suicide between 1971 and 2003. With Cox Proportional Hazards models, we assessed the effects of body size at birth (birth weight, and length, head circumference, and placental weight at birth) and length of gestation on the risk of suicide, stratifying for year of birth and adjusting for socioeconomic position in childhood in all analyses, and for length of gestation when assessing the effects of body size at birth.

In women, larger head circumference (HR = 1.94, 95% CI= 1.32-2.85) and longer length at birth (HR= 1.63, 95% CI= 1.05-2.54) significantly predicted an increased risk of suicide. Higher birth weight (HR= 1.53, 95% CI= 0.99-2.38) was marginally associated with increased risk. In contrast, in men, length of gestation showed a quadratic effect on the risk of suicide (p=.03). Boys born preterm (< 37 weeks) were at a 2.59-fold (95% CI 1.37-4.90) increased risk of suicide in comparison to term-born (37-42 weeks) boys. Further adjusting for maternal height and BMI at childbirth rendered the effect of birth length on suicide risk among women marginally significant (p=0.052), but did not change the other findings.

Our findings suggest sex-specific prenatal programming effects on the risk of suicide. While among women suicide risk was predicted by rapid prenatal growth; among men, preterm birth emerged as a risk factor for suicide.

PII-123

Prevalence and Perinatal and Early Life Factors Associated with Depression in Brazilian Children. Thaís Pereira[1], Antônio Silva[1], Bettiol Heloisa[2], Barbieri Marco[2], Rodriguez Juliana[1]. *[1]Public Health, Federal University of Maranhão, Maranhão, Brazil; [2]Department of Pediatrics, Faculty of Medicine of Ribeirão Preto, São Paulo, Brazil.*

To estimate the prevalence and perinatal and early life factors associated with depression in children aged seven to 11 years from two Brazilian birth cohors.

The study was based on two birth cohorts, one initiated in Ribeirão Preto (RP) in 1994 and another in São Luís (SL) in 1997-98 and in follow-up studies conducted when children were nine to 11 years in RP in 2004/05, and seven to nine years in SL, in 2005/06. In the first step in RP, included 2911 newborns in a period of four months, representing 99% of all live births. The final sample consisted of 2846 births. In SL this stage was conducted from March 1997 to February 1998, including 2542 births, which accounted for 96.3% of all born in the period. The final sample consisted of 2443 births. Follow-up rates were 69.1% in RP (790 participants) and 72.7% in SL (673 participants). To investigate the depression we used the Child Depression Inventory, a sub-sample of 774 children in RP and 670 in SL at school age. The depression variable was categorized into yes (CDI ≥ 17) and not (CDI <17). Adjusted and non-adjusted prevalence ratios (PR) were estimated by Poisson regression.

The prevalence of depression was 7.2% in RP and 21.0% in SL. In non-adjusted analysis in SL, maternal education> 9 years and high income were protective factors and parental educational level <9 years was a risk factor for depression. In RP, one to four household members and family income were protective factors and parental educational level <4 years, low birth weight, intrauterine growth restriction and skilled manual occupation, semi-skilled, unskilled and unemployed head of family were risk factors for depression. In adjusted analysis, in RP, birth weight of 500-1499g (PR=5.56), of 1500-2499g (PR=2.94), skilled and semi-skilled occupation (PR=3.63) and unskilled occupation and unemployment (PR=3.21) were risk factors for depression. In SLv, paternal schooling of 0-4 years (PR=2.19) and of 5-8years (PR=1.71) were independent risk factors for depression.

The prevalence of depression was much higher in the less developed city, SL, than in RP and than prevalences reported in several international studies. Low socioeconomic level was associated with depression in both cohorts. Low birth weight was a risk factor for depression in RP.

PII-124

Influence of Birth Conditions and Catch-Up Growth in Weight on Pulmonary Function in Brazilian Children: A Cohort Study. Maria L.V. Figueiredo[1], Antonio A.M. Silva[1], Elcio S.O. Vianna[2], Heloisa Bettiol[2], Marco C. Barbieri[2], Nathalia A.C. Silva[1], Valdinar S. Ribeiro[1], Maria R.S.R. Ribeiro[1]. *[1]Federal University of Maranhao, Brazil; [2]Faculty of Medicine of Ribeirao Preto - University of Sao Paulo, Brazil.*

To evaluate the association of low birth weight (LBW), preterm (PT) birth and intrauterine growth restriction (IUGR) with pulmonary function in children aged seven to 11 years in two Brazilian birth cohorts: Ribeirão Preto (RP) and São Luís (SL).

The study was conducted on 694 children belonging to two cohorts whose information was collected at birth and school age in 1994 and 2004/05 in RP (n=362) and in 1997/98 and 2005/06 in SL (n=332). Lung function was measured by basal spirometry (FEV1 and FVC). Values for each lung function measurement were expressed as standard deviation scores. Birth weight ratio (BWR) < 0.85 was defined as IUGR, as proposed by Kramer. Catch-up growth in weight was defined as an increase of at least 0.67 z-score. The coefficients and confidence intervals for FEV1 and FVC according to LBW, PT, IUGR and catch-up growth in weight adjusted for confounding were estimated by linear regression in separate models for each city.

In RP, in univariable analysis, very low birth weight showed a significant association with reduced FEV1 (P=0.01). Preterm newborns with IUGR also showed a significant reduction in FEV1. Consensual union, family income of 1-9 minimum wages, non-white skin color, mechanical ventilation, asthma, bronchitis and wheezing were associated with reduced FEV1. After adjustment, in RP, asthma, bronchitis, wheezing, family income of 1-3 minimum wages and preterm children with IUGR continued to be negatively associated with FEV1. Catch-up growth was positively associated with FEV1 (P=0.02). In SL, in uni and multivariable analysis, only previous hospitalization due to pneumonia was associated with reduced FEV1 and CVF. In RP, in univariable analysis, low family income and non-white skin color were associated with reduced values of FVC. Also in RP, catch-up growth in weight was positively associated with FVC, in uni and multivariable analysis.

In RP neither PTB nor IUGR were associated with lung function. Only PTB in combination with IUGR was associated with reduced FEV1 whereas catch-up growth in weight was associated with increased FEV1 and FVC. High mortality in the VLBW group may explain why no association between PTB, IUGR and catch-up growth in weight with both FEV1 and FVC was detected in SL.

PII-125

Gene Expression and Adult Cardiac Wall Thickening Following Prenatal-Postnatal Nutrient Mismatch in Sheep. Kirsten R. Poore, Shafaf A. Shahruddin, Frederick W. Anthony, Jane K. Cleal, Mark A. Hanson, Lucy R. Green. *Institute of Developmental Sciences, University of Southampton, United Kingdom.*

Left ventricular (LV) and intraventricular septum (IVS) thickening after prenatal undernutrition in adult male sheep is reduced when the prenatal-postnatal nutrient mismatch is minimized (Cleal *et al.*. 2007 PNAS 104, 9529-33). This effect is sex-specific and a molecular basis was determined by measuring expression of oestrogen receptor alpha (ER-α, conferring cardiovascular protection and anti-cardiac hypertrophy), type 1 angiotensin II receptor (AT1R, implicated in cardiovascular disease and cardiac hypertrophy) and Brain Natriuretic Peptide (BNP, anti-fibrotic and anti-hypertophic).

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Welsh Mountain ewes received 100% (C, *n*=34) or 50% of nutrient requirements (U, *n*=35) from 1-31 days of gestation, and 100% thereafter. Offspring were fed *ad libitum* (CC, *n*=20; UC, *n*=13) or to reduce body weight to 85% of individual target weight from 12 to 25 weeks postnatal age and *ad libitum* thereafter (CU, *n*=14; UU, *n*=22). At 2.5 years ER-α, AT1R and BNP mRNA levels were measured in LV by semi-quantitative RT-PCR relative to the geometric mean of RPL19 and βActin housekeeping genes. Data were analysed by ANOVA and linear regression.

There was no effect of prenatal or postnatal diet, offspring sex or twinning on ER-α, AT1R and BNP mRNA levels. ER-α and AT1R mRNA levels were correlated across all diet groups in males ($P<0.0001$, $r^2=0.81$) and females ($P<0.0001$, $r^2=0.82$). In females only ER-α ($P<0.05$, $r^2=0.13$) and AT1R ($P<0.05$, $r^2=0.19$) mRNA levels were correlated with mean arterial blood pressure. BNP mRNA and IVS thickness tended to be correlated across all diet groups in males only ($P=0.08$, $r^2=0.12$), significantly in CC ($P<0.05$, $r^2=0.6$). In females, BNP mRNA and IVS thickness were negatively correlated (all groups: $p<0.05$, $r^2=0.19$).

These data support a link between oestrogen and the renin angiotensin systems, and suggest that their relative importance in regulating baseline blood pressure may be sex-specific. BNP was a weak marker of ventricular thickness in a sex-dependent manner, and may only contribute significantly to later fibrotic stages of ventricular hypertrophy. Overall, our findings do not support a role for these genes in mediating the sex-specific effects of prenatal-postnatal nutrient mismatch on ventricular thickening.

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PII-126

Fetal Cardiac Autonomic Control during Breathing Movements: The Effect of Maternal Exercise. Kathleen M. Gustafson[1], Linda E. May[2], Hung-wen Yeh[3]. [1]*Neurology, University of Kansas Medical Center, KS, USA;* [2]*Anatomy, Kansas City University of Medicine and Biosciences, MO, USA;* [3]*Biostatistics, University of Kansas Medical Center, KS, USA.*

Using a dedicated fetal biomagnetometer, we identified and characterized the fetal diaphragmatic magnetomyogram (dMMG) generated during breathing movements and reported the effects on fetal cardiac autonomic control during breathing and non-breathing movements (apnea). We next used this method to investigate fetal heart rate (HR) and heart rate variability (HRV) during fetal breathing movements and periods of apnea in two groups of women: those who exercised during their pregnancy and those who did not.

30 healthy, pregnant women consented to the study. 15 women were classified as exercisers and 15 as non-exercisers (Control) based on a physical activity questionnaire. At 36 weeks gestational age, we used a CTF System, 83 channel biomagnetometer to record the magnetocardiogram (MCG). Raw data were recorded using a 300 Hz sampling rate and recording filter of 0-75 Hz. Data were digitally filtered (1-40 Hz) and then underwent independent component analysis (ICA) using EEGLab (v4.311) in order to segregate the contributions from spatially distinct electrophysiological sources into individual components. Epochs of fetal dMMG of one min or longer were matched to an apneic period in the same recording. Fetal MCG R-waves were marked and fetal HR and HRV were analyzed in each dataset (2/fetus).

Fetal breathing movements had a significant effect on fetal HR, overall HRV (SDNN), short-term HRV (RMSSD), Log RSA, and high frequency power (HF). There were significant group effects with lower fetal HR and higher fetal HRV in the Exercise Group and significant group by condition interactions for the putative parasympathetic metrics.

Fetal HR is lower during both breathing and apneic periods and parasympathetic input is higher during breathing movements in the exercise group when compared to the non-exercising cohort. Maternal exercise during pregnancy has an effect on fetal cardiac autonomic control and could potentially influence long-term cardiac health and adaptability in the offspring.

PII-127

Inhalation of Hyperoxic Gas in the Neonatal Period Has Long-Term Effects on the Pulmonary Airways. Megan O'Reilly[1], Philip Hansbro[2], Jay Horvatt[2], Foula Sozo[1], Richard Harding[1]. [1]*Anatomy and Developmental Biology, Monash University, VIC, Australia;* [2]*Hunter Medical Research Institute, NSW, Australia.*

Preterm infants often require prolonged support with high levels of inspired oxygen. Very preterm birth is associated with later impairment of lung function, but the role of neonatal exposure to hyperoxia on conducting airways is poorly understood. Our aim was to determine the structural and functional effects of exposing the immature lung to hyperoxic gas in adulthood.

Neonatal mice (C57Bl/6J) were continuously exposed to hyperoxic gas (65% O_2) from birth until postnatal day 7 (P7d, n=15). Following the hyperoxia-exposure (H-E) period, mice lived in room air (21% O_2) until adulthood at 10 months of age (P10mo). Controls breathed room air from birth to P10mo (n=16). At P10mo, lung function was assessed by measurement of transpulmonary resistance and dynamic compliance in response to increasing doses of the bronchoconstrictor methacholine using whole-body invasive plethysmography. Bronchoalveolar lavage fluid (BALF) was collected and the immune cells in BALF counted. After necropsy (P10mo), the structure of the bronchioles and lung parenchyma was analysed morphometrically in lungs fixed at 25 cmH_2O.

At P10mo, H-E mice had significantly more smooth muscle in the outer bronchiolar wall compared to controls ($p<0.05$) and fewer alveolar-bronchiolar attachments ($p<0.05$). In the lung parenchyma there was a greater mean linear intercept (indicative of larger alveolar diameter), a smaller percentage area of tissue and more airspace (all $p<0.05$). In response to increasing doses of methacholine, H-E mice tended to have a smaller increase in pulmonary resistance ($p=0.083$) and a smaller decrease in dynamic compliance ($p=0.099$) compared to controls. At the highest methacholine dose (20mg/ml) H-E mice had significantly greater lung compliance ($p<0.05$) than controls. H-E mice had 90% more immune cells in BALF than controls ($p<0.05$); in both groups, >95% of immune cells were macrophages.

Inhalation of hyperoxic gas in the neonatal period results in persistent structural alterations of the bronchioles and lung parenchyma in "middle-age". These structural alterations are likely to contribute to the altered lung function seen at P10mo. The presence of increased numbers of immune cells within the lung may indicate low-grade chronic inflammation and may affect the ability of the lungs to clear pathogens.

PII-128

Intra-Uterine Inflammation as a Modifier of Fetal Lung Development: Do Effects Persist after Preterm Birth? Anzari Atik, Robert De Matteo, Foula Sozo, Takushi Hanita, Richard Harding. *Anatomy and Developmental Biology, Monash University, Australia.*

Intra-uterine inflammation is now considered to be a cause of preterm birth and may also affect fetal lung development. Recent studies indicate that prolonged fetal exposure to intra-uterine inflammation induced by lipopolysaccharide (LPS) may stimulate pulmonary surfactant production but inhibit alveolarization. However few studies have examined the postnatal persistence of the effects of intra-uterine inflammation on lung structure and surfactant production. Inflammation could contribute to altered lung development in preterm infants. Our aim was to determine the effects of chronic intra-uterine inflammation on lung structure and pulmonary surfactant protein gene expression in postnatal lambs following preterm birth.

Fetal sheep were exposed to LPS (1 mg/day, n=6) or saline (n=9) infused into the amniotic sac from 110 days of gestation until induced preterm birth at ~133 DGA (term ~147d). After birth, lambs were raised with their mothers until necropsy at 11 weeks postnatal age. At necropsy one lung was pressure-fixed for structural analysis and the other frozen for assessment of surfactant protein gene expression by qRT-PCR.

There were no differences between groups in either birthweight or postnatal weight gain. In the lung parenchyma, we found no differences between groups in percentages of tissue and airspace, alveolar size (mean linear intercept), elastin deposition and collagen deposition. In the bronchiolar walls, we found no significant differences in the relative amounts of

epithelium, collagen and airway smooth muscle. The number of bronchiolar-alveolar attachments was unaltered. However, surfactant protein (SP)-A and SP-C mRNA levels were significantly higher in LPS-exposed lambs compared to controls (both $p<0.05$); SP-B and SP-D were not different between groups. In LPS-exposed lambs, the weights of the spleen and kidney, relative to body weight, were respectively 42% and 13% lower than in controls (both $p<0.05$).

Prenatal LPS exposure does not lead to persistent structural changes in the lung parenchyma or bronchioles. However it causes a persistent increase in surfactant protein gene expression, which could reflect on-going inflammation. The decreases in kidney and spleen weights could influence disease susceptibility in later life.

PII-129

Maternal Dietary Fat during Pregnancy and Lactation Impairs Vascular Function of Adult Offspring. Christopher J. Kelsall[1], Nicola A. Irvine[1], Christopher Torrens[1], Karen A. Lillycrop[2], Mark A. Hanson[1], Philip C. Calder[1], Graham C. Burdge[1]. *[1]Faculty of Medicine, University of Southampton, Hampshire, United Kingdom; [2]Faculty of Natural and Environmental Sciences, University of Southampton, Hampshire, United Kingdom.*

Human populations exhibit temporal and cross-sectional variations in fatty acid intake. Variations in dietary saturated (SFA), polyunsaturated (PUFA) and *trans* fatty acid (TFA) intakes altered cardiovascular disease risk in humans[1]. Maternal high saturated fat diet during pregnancy and lactation in rats is associated with impairment of vascular function in offspring[2], but it is not known whether differences in the type of maternal dietary fat influence future cardiovascular function in the offspring.

To investigate this, female rats were fed either 7% (w/w) or 21% (w/w) safflower oil (SAO, enriched in linoleic acid), hydrogenated soybean oil (HSO, enriched in TFA), butter (enriched in SFA) or fish oil (FO, enriched in eicosapentaenoic and docosahexaenoic acids) from two weeks prior to mating until offspring were weaned at day 28 onto AIN93M (4% w/w soybean oil). *Ex-vivo* resistance artery endothelial function (relaxation of mesenteric arteries to acetylcholine; ACh, 0.1 nM - 10 μM) was measured in 77 day old offspring by wire myography. Statistical analysis was performed using a general linear model with Tukey's *post hoc* testing; significance was ascribed at $p<0.05$. Data are % relaxation, mean±SD.

There were significant effects of the type of maternal dietary fat (p 0.012), total maternal dietary fat (p 0.001), and an interaction between sex and type of maternal dietary fat (p 0.008) on ACh-induced relaxation of mesenteric arteries. Vasorelaxation to 10 nM ACh was impaired in male 21% butter (-0.2±7.5) and FO (6.2±6.5) offspring compared to 7% butter (18.8±19.3) or FO (38.5±16.1) offspring, and in female 21% butter (5.0±8.4) compared to 7% butter (42.7±35.4) offspring.

The data show that both type and amount of maternal dietary fat induce persistent impairment in the vascular function of the offspring contingent on the sex of the offspring.

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1. Reddy KS (2002). *Public Health Nutr* **5**, 231-7
2. Khan IY *et al.* (2004). *Am J Phys* **288**, R127-33

PII-130

Hypoxia-Like Gene Responses to Maternal Undernutrition in Fetal Heart. Yoshitaka Kimura[1], Takuya Ito[2], Kaori Tanabe[1], Ai Nakamura[1], Kiyoe Funamoto[1], Ayako Aoyagi[3], Kazuyo Sato[3], Tetsuro Hoshiai[3], Kaori Suenaga[3], Junichi Sugawara[3], Satoru Nagase[3], Nobuo Yaegashi[3], Kunihiro Okamura[4]. *[1]International Advanced research and Education Organization, Tohoku University, Japan; [2]Innovation of New Biomedical Engineering Center, Tohoku University, Japan; [3]Division of Obstetrics and Gynecology, Tohoku University, Japan; [4]KKR Tohoku Kousai Hospital, Japan.*

It is known that maternal undernutrition during pregnancy is a factor inhibiting fetal growth and inducing cardiovascular diseases. However, the mechanism is complicated and still unknown. We have designed an experimental model of undernutrition in pregnant mice to evaluate the gene background in murine fetal heart by maternal undernutrition.

Female C57BL/6N mice were given low protein (L) or regular (N) food two weeks before mating and during their pregnancy. The fetal hearts were collected on day 17.5 of gestation, which was late pregnancy, to evaluate effercts of maternal undernutrition on overall gestation period.

Fetal hearts were analyzed comprehensively by microarray. In addition, genes of interest (hypoxia-inducible factor 1 alpha: HIF1a and prolyl hydroxylases: PHD1, HIF1a inhibitory regulator) were assayed by quantitative real-time PCR (qPCR), and evaluated in protein levels of HIF1a by immunohistochemistry.

Comprehensive microarray analysis showed 133 transcription factors changed by maternal undernutrition. Among them, HIF1a that is an important transcription factor was significantly increased by maternal undernutrition. qPCR showed the mRNA expression levels of both HIF1a and PHD1 of male fetal heart were increased by maternal undernutrition. In addition, immunohistochemical analysis showed the protein level of HIF1a was increased by maternal undernutrition.

Increase in the expression of HIF1a is seen in hypoxia. In contrast, increase in HIF1a in tumor has been reported even in normoxia. In this study, HIF1a was increased without hypoxia in fetal heart by maternal undernutrition suggesting that maternal undernutrition induced hypoxia-like gene responses in fetal heart.

PII-131

Global DNA Methylation during Pregnancy and Asthma at Age 7 Years. Nancy E. Lange[1], Matthew W. Gillman[2], Sheryl Rifas-Shiman[2], Diane R. Gold[1], Letizia Tarantini[3], Andrea Baccarelli[4], Augusto A. Litonjua[1]. *[1]Brigham and Women's Hospital, USA; [2]Harvard Medical School/Harvard Pilgrim Health Care Institute, USA; [3]University of Milan, Italy; [4]Harvard School of Public Health, USA.*

Asthma, one of the most common chronic conditions of childhood, likely originates *in utero*. DNA methylation patterns, which affect gene expression, begin to be established in early development. We examined associations of global DNA methylation during pregnancy and at delivery with asthma-related outcomes at age seven years.

In the pre-birth cohort study Project Viva, we measured percent methylation of long interspersed nuclear element-1 (LINE-1) in white blood cells from maternal blood in the 1st trimester (n=667) and 2nd trimester (n=676), and cord blood collected at delivery (n=416). Our main outcome ("asthma") was doctor-diagnosed asthma at age seven plus either use of asthma medication or wheezing in the past 12 months. Our secondary outcome was airway obstruction, measured as FEV_1/FVC from pulmonary function testing. We used logistic regression adjusted for maternal education, race/ethnicity, smoking during pregnancy, parental asthma, household income, gestational age at birth, child age, and (for FEV_1/FVC) height; and stratified by sex.

Mean (SD) LINE-1 methylation levels were 84.3 (0.6), 84.5 (0.4), and 84.6 (0.6) %5mC for 1st trimester, 2nd trimester, and cord blood, respectively. LINE-1 methylation was higher among boys than girls in cord blood (84.8 v 84.4, $p<0.0001$) but not in maternal blood. 64 (14.9%) girls and 87 (21.0%) boys had asthma at age 7. In adjusted analysis, each 1% increment in 1st trimester LINE-1 methylation was associated with increased odds of asthma at age seven among boys (OR 2.17 [95% CI 1.33, 3.53]) but not girls (0.97 [0.56, 1.66]). Consistent with this finding, each 1% increment in 1st trimester LINE-1 methylation was associated with a 3.2% decrease (95% CI -5.8, -0.7) in the FEV_1/FVC among boys but not among girls (0.4 [-1.9, 2.6]). We did not find consistent associations of 2nd trimester or cord blood LINE-1 with asthma or airway obstruction among boys or girls.

Higher 1st trimester LINE-1 methylation was associated with increased odds of asthma and with airway obstruction at age seven among boys only. These findings raise the possibility that effects of maternal DNA methylation in early pregnancy may explain the observed sex differences in the incidence and prevalence of asthma in school-age children.

PII-132

Endothelial Progenitor Cells Dysfunction in Low Birth Weight Infants: A Possible Involvement of Circulating Inhibitors. Isabelle Ligi[1,2,3], Edwige Tellier[2,3], Stéphanie Simoncini[2,3], Francine Anfosso[2,3], Françoise Dignat-George[2,3,4], Umberto Simeoni[1,2,3]. *[1]Neonatal medecine department, Assistance Publique Hôpitaux de Marseille, Marseille Cedex 5, France; [2]Laboratoire de physiopathologie de l'endothélium UMR 608, INSERM, Marseille, France; [3]Laboratoire de physiopathologie de l'endothélium, Faculté de Pharmacie, Université Aix-Marseille, Marseille, France; [4]Laboratoire d'Hématologie, Assistance Publique Hôpitaux de Marseille, Marseille cédex 5, France.*

Low birth weight is a risk factor for hypertension and coronary heart disease at adulthood. Early endothelial dysfunction has been characterized in low birth weight (LBW) infants. Emerging *in vitro* data show that endothelial angiogenic function is altered in LBW infants; however, the mechanisms are unknown. We hypothesized that changes in soluble, plasma pro- or anti-angiogenic factors are associated with endothelial dysfunction and impaired angiogenesis in LBW infants.

Venous umbilical cord blood was collected from 42 normal, term, control babies and 75 low birth weight babies. Endothelial progenitor cells (EPC) from control patients were cultured in the presence of 10% of serum obtained from both groups. Cell proliferation, migration and invasion ability was measured using a cell proliferation kit, wound healing method and the Matrigel invasion assay. ELISA immuno-assays for human vascular endothelial growth factor (VEGF), soluble VEGF receptor (sVEGFR), endoglin were performed on umbilical cord blood samples.

The proliferation and the migration of EPCs were significantly reduced when cultured with 10% of serum of low birth weight babies compared to serum of control babies. Matrigel invasion assay was not significantly different between both. Umbilical vein plasma VEGF concentration was significantly reduced in low birth weight babies while that of sVEGFR was significantly higher. The endoglin level was not different between the groups. Addition of VEGF corrected the inhibitory effect of LBW serum on normal EPCs proliferation.

Serum obtained from low birth weight babies may contain factors that exhibit an antiangiogenic effect on EPC proliferation and migration. VEGF/sVEGF pathway seems to be involved in the endothelial dysfunction associated with the developmental programming of hypertension at adulthood.

PII-133

IGF-1 Does Not Restore Cell Cycle Activity in Fetal Sheep Cardiomyocytes Following Placental Insufficiency. Samantha Louey[1,2], Natasha N. Chattergoon[1], Sonnet S. Jonker[1,2], George D. Giraud[1,2,3], Kent L. Thornburg[1,2]. *[1]Heart Research Center, Oregon Health & Sci Univ, USA; [2]Dept Med (Cardiovasc Med), Oregon Health & Sci Univ, USA; [3]Portland VA Med Ctr, USA.*

Placental insufficiency induced by umbilicoplacental embolization (UPE) in fetal sheep suppresses cardiomyocyte cell cycle activity and maturation (Louey *et al.*, 2007), but the capabilities of the heart to recover from this insult are not known. The aim of this study was to determine the degree to which an undergrown heart can increase cell cycle activity (and hence cell number) in response to IGF-1, a known stimulant of cardiomyocyte proliferation.

Fetal sheep were studied between 115 and 130 days gestational age (dGA, term ~145d GA): controls (n=4), 15d UPE (n=6), 10d UPE followed by 5d IGF-1 (UPE-IGF1, n=6). During UPE, daily injections of nonsoluble microspheres into the umbilicoplacental circulation induced fetal hypoxemia. We have shown 715μg/day (~6.6μg/kg/hr) Long R3 IGF-1 increases cardiomyocyte proliferation (Sundgren *et al.*, 2003); given decreased body weight in UPE fetuses a lower dose (560μg/day, 6.7±0.3μg/kg/hr i.v.) was used in the current study. Postmortem, fetal hearts were enzymatically dissociated. Isolated cardiomyocytes were analyzed for maturational state (proportion that are binucleated) and cell cycle activity (Ki-67). For clarity only left ventricular data are reported, data are mean±SEM.

After 10d, all UPE fetuses were hypoxemic (PaO2: 14±0.5mmHg vs 22±2mmHg, $p<0.01$) and hypoglycemic (0.7±0.1mmol/L vs 0.9±0.1mmol/L, $p<0.05$) versus controls. UPE-IGF1 fetuses remained hypoxemic (17±1mmHg, $p<0.05$) despite no UPE during the final 5d of study, and hypoglycemia worsened (0.2±0.1mmol/L, $p<0.01$). Body weights of 15d UPE fetuses were 24% less ($p<0.05$) than control and UPE-IGF1 fetuses; heart weights (g/kg body) were not different between groups. As previous, 15d UPE suppressed cardiomyocyte cell cycle activity (3±1%, $p<0.01$) and binucleation (35±2%, $p<0.05$) compared to controls (9±1% Ki-67 positive; 53±5% binucleation). IGF-1 therapy did not restore cell cycle activity (5±1%, $p<0.05$) and only partially restored % binucleation (41±5%).

IGF-1 failed to ameliorate the negative effects of placental insufficiency on cardiomyocyte growth. Perhaps of greater concern is the worsening of hypoglycemia in response to IGF-1 despite the cessation of daily embolization. We conclude that normal IGF-1 signaling is disrupted under conditions of placental insufficiency.

PII-134

Metabolic Syndrome and Subclinical Atherosclerosis in Children. Pilar Arnaiz, Luis Villarroel, Ivan Godoy, Salesa Barja, Oscar Castillo, Marcelo Farias, Angelica Dominguez, Francisco Mardones. *Pontificia Universidad Catolica de Chile, Santiago, Chile.*

Atherosclerosis begins in childhood in response to the clustering of metabolic syndrome (MS) risk components since early life. Carotid intima-media thickness (CIMT), a surrogate marker of subclinical atherosclerosis, has been strongly related with cardiovascular disease and Diabetes 2 in adulthood. We aimed to study the possible association of CIMT with the MS components in a population of Chilean children.

A cross-sectional study of 304 children of low socio-economic strata from an urban area of Santiago was performed during 2009-2010. This sample was selected mainly considering the presence of one or more MS components and insulin resistance (IR). Anthropometry and systolic and diastolic blood pressure were assessed by trained personnel. While fasting, a blood sample was taken to determine lipids (enzymatic colorimetric tests), glycemia (hexoquinase), insulin (quimioluminiscence) and HOMA. CIMT was assessed using ultrasonography with automated software. Pearson correlation, chi-squared test and stepwise regression were used.

Mean age was 11.5 ± 0.9 years old; 57% girls and 42% pre-pubertal; 64% overweight; and 25% had MS. MS components distribution was: 20% had 0, 29% had one, 26% had two and 25% had three or more. Pearson coefficients for CIMT medium and maximum with systolic blood pressure were: 0.206 (p 0.0003) and r: 0.213 (p 0.0002). Some MS components and IR had higher proportions of children over the 75th percentile of CIMT medium and maximum in the contingency tables: 1) the proportion of children with high CIMT medium was significantly higher for IR (0.035) and CHDL ≤ 40 mg/dL (p 0.039); 2) A tendency for significant differences was found for the proportions of children with high (>75th percentile) CIMT medium with waist circumference (p 0.052) and children with high (>75th percentile) CIMT maximum with CHDL ≤ 40 mg/dL (p 0.062). Multiple linear regression models for CIMT medium and maximum selected just systolic blood pressure in both; p values were 0.0003 and 0.0002.

Univariate analyses showed that CIMT medium and maximum were directly associated with systolic blood pressure. Bivariate analyses showed that CIMT medium was significantly associated with IR and CHDL ≤ 40 mg/dL. However, multiple regression models for both CIMTs just selected systolic blood pressure, a fact that would make it a proxy for CIMTs. These results are the first reported in our country.

PII-135

Pregnancy Activity Level Influences Infant Heart Outcomes. Linda E. May[1], Kathleen M. Gustafson[2], Hung-wen Yeh[3], Richard S. Suminski[1]. *[1]Department of Physiology, Kansas City University of Medicine and Biosciences, MO, USA; [2]Department of Neurology, University of Kansas Medical Center, KS, USA; [3]Department of Biostatistics, University of Kansas Medical Center, KS, USA.*

We have shown that maternal leisure time physical activity (LTPA) during pregnancy results in lower fetal heart rate (HR) and higher fetal heart rate variability (HRV) and the results depend on the LTPA intensity and energy expenditure (i.e., dose response). Our next goal was to determine if the dose response relationship between maternal LTPA and fetal cardiac autonomic control was an in utero phenomenon or whether these findings persist into the postnatal period.

Relationships between infant HR and HRV and maternal LTPA measures (n=42 pairs) at one month postnatal age were assessed by Spearman

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correlation. Further regression analysis of significant fetal-maternal correlations was performed to control for maternal covariates (maternal age, pre-pregnancy BMI, maternal weight gain, maternal resting HR).
Mean maternal age was 29 years and the median pre-pregnancy BMI was healthy (23.9) with median weight gain of 14.1 lbs. Mean maternal resting HR was 90 bpm, and women expended about 90 kcal/day participating in LTPA. Maternal LTPA energy expenditure significantly predicted infant low frequency power (p=0.01) HRV. Maternal LTPA energy expenditure significantly predicted short-term HRV (p=0.03), and high frequency power (p=0.03) which are two metrics dominated by parasympathetic input.
Maternal LTPA energy expenditure dose during pregnancy results in beneficial changes in fetal HR and HRV that are still present at one month postnatal age. We conclude that the dose of regular maternal LTPA during gestation influences infant autonomic control training response; further, LTPA during pregnancy is the earliest time period to influence offspring cardiac health.

PII-136

The impact of Assisted Reproduction Therapy on Infant Mortality in Porto Alegre, Brazil. Marilyn Agranonik[1,2,3], Marjori Zanetello[1,2,3], Marcelo Goldani[1,2,3], Patrícia Peluffo Silveira[1,2,3], Clécio Homrich da Silva[1,2,3]. *[1]Universidade Federal do Rio Grande do Sul, Brazil; [2]Hospital de Clínicas de Porto Alegre, Brazil; [3]Núcleo de Estudos da Saúde da Criança e do Adolescente (NESCA), Brazil.*
Multiple births are associated with infant morbidity and mortality. There has been an increase in the rate of multiple births in developed countries, probably related to dizygotic twins and late pregnancies. The objective of this study was to investigate the impact of assisted reproductive technologies related to multiple births in infant mortality and its components.
This is a registry-based study. Data was obtained from birth and death certificates of all live births in Porto Alegre, a medium-sized developed city in southern Brazil, from 1996 to 2007. Newborns weighing less than 500g were excluded. Weinberg's equation was used to calculate the proportion of monozygotic (MZ) and dizygotic (DZ) twins. Chi-squared for trend was used to evaluate trend of infant mortality rates in MZ and DZ twins during the period. Population Attributed Risk (PAR) has been used to assess the impact of DZ and MZ twins in the infant mortality rates.
The overall infant mortality rates declined during the period. The multiple births rate increased from 1.95% in 1996 to 2.17% in 2007 (p=0.01). Among the twins, DZ rates increased from 59% to 77% (p <0.01). Infant mortality rates of DZ remained stable (p> 0.05), while among MZ decreased (p <0.01). Considering all live births (multiple and singletons), in 1996, 1.6% of infant mortality rate and 1.9% of neonatal mortality rate were attributed to DZ twins, while in 2007 this rates increased to 2.6% and 6.5%, respectively.
We suggest that ART influenced the increase of DZ rates leading to negative impact on infant mortality. Thus, we can infer that the ART helps to slow the decline in infant mortality rates. Although there have been advances in perinatal and neonatal care, public policies are becoming necessary for the management of new health technologies.

PII-137

Comparison of 'Birth Weights' Taken by MMNP Team and Hospital Medical Records in an Ongoing RCT in Urban Slums of Mumbai. Sujay Joshi[1], Ramesh Potdar[1], Meera Gandhi[1], Nivedita Nikam[1], Monica Dayama[1], Caroline Fall[2]. *[1]Center for the Study of Social Change, Mumbai, India; [2]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom.*
To compare the measures of birth weight taken by the MMNP team to the data obtained in the hospital medical records.
Mumbai Maternal Nutrition Project (MMNP) is an ongoing randomized controlled trial (RCT) of food based micro-nutrient supplementation in slum women with the primary outcome of increase of birth weight of newborns by 100 gm.
Since 2006, a total no. of 6772 women registered and around 1300 babies are born under the trial. The babies are measured for their birth weight by visiting the Hospitals they were delivered within 72 hrs of their birth. The Birth weight is measured by using an Electronic Weighing Scale (MMNP birth weight). Monthly calibration is done of these electronic weighing scales for maintaining the quality and accuracy of the birth weight data.
Out of 1300 babies born under trial, some of the babies 'birth weight' measurements are missed due to various reasons like women delivered at village, out-migration and other such factors. Since some of the babies 'birth weight' data is missed, to see whether we can use their hospital recorded birth weight we thought of comparing the birth weight taken by MMNP team and recorded by hospital staff.
By means of an electronic weighing scale , the birth weight of the babies were being measured. For the same babies the data from the hospital medical records taken by the hospital staff was also obtained.
We compared the MMNP birth weight and Hospital recorded birth weight for 54 babies. It was found that, for each 1 kilogram of MMNP birth weight, hospital birth weight was over measured by 10 grams.[n=54, B=1.01, 95% CI =(0.94, 1.08) p=<0.001]. This over measurement in terms of the birth weight was found to be higher in public sector hospitals in comparison to privately owned hospitals.
Hospital recorded birth weights appear to be more by 10gms/kg in comparison to the MMNP birth weight. For those babies whose birth weight could not be measured by MMNP team, it may be possible to use their hospital birth weight data by making statistical adjustments. The pros' and cons' of using of hospital birth weight record measurements for analysis will be discussed in the presentation.

PII-138

Markers of Early Cardiovascular Damage in Young Indian Adults: Pune Children Study. A. Kinare[1], S. Joshi[1], S. Hardikar[1], S. Wagle[1], V. Kantikar[1], H. Lubree[1], D. Bhat[1], L. Ramdas[1], A. Pandit[1], C. H.D. Fall[2], C. Yajnik[1]. *[1]Diabetes and Pediatrics Unit, KEM Hospital Research Centre, Pune, India; [2]MRC Lifecourse Epidemiology Unit, Southampton General Hospital, University of Southampton, Southampton, United Kingdom.*
Intima media thickness (IMT), pulse wave velocity (PWV) and arterial stiffness index (ASI) are some of the non-invasive measurements of vascular damage. We measured these indices in participants of Pune Children Study, a birth cohort from urban Pune, and studied their association with some of the known cardiovascular risk factors.
PWV and ASI (Periscope cardiovascular analyzer, Genesis Medicals Systems Pvt Ltd, India) were measured in 324 participants at 21 years of age (175 males, 149 females). IMT (Pro-sound α-7, Aloka, Japan) measurements were available in 191 participants, Anthropometric measurements, blood pressure and biochemical-metabolic measurements were performed by standard methods.
Participants were 59.4±13.6 kg and 165.6±9.5 cm. Twenty two were hyperglycemic, seventeen were dislipidemic and ten were hypertensive. PWV, ASI and IMT were positively interrelated, and all were higher in males (p<0.05). PWV and ASI were positively related to systolic blood pressure (R^2 9.7% and 12.9% respectively), ASI was inversely related to diastolic blood pressure and triglycerides (R^2 2.4% and 3.9% respectively) ; both were not related to birth weight or current weight. IMT was directly related to current weight (R^2 2.5%) and inversely with glycemia (R^2 3.4%).
Our study has measured markers of early vascular damage in young adults in a birth cohort. These measurements will be useful in studying evolution of cardiovascular disease in this cohort.

PII-139

The Changing Face of Gestational Diabetes: The FinnGeDi Study. Sanna Klemetti[1,2], Eero Kajantie[2,3], Jatta Pirkola[1], Hannele Laivuori[4], Risto Kaaja[5], Johan G. Eriksson[2,6], Mika Gissler[2], Aini Bloigu[2], Anneli Pouta[2], Marja Vääräsmäki[1,2]. *[1]Department of Obstetrics and Gynecology, Oulu University Hospital, Finland; [2]National Institute for Health and Welfare, Oulu and Helsinki, Finland; [3]Children´s Hospital, University of Helsinki, Finland; [4]Department of Medical Genetics, University of Helsinki, Finland; [5]Satakunta Central Hospital, University of Turku, Pori, Finland; [6]Department of General Practice and Primary Health Care, University of Helsinki, Finland.*
Gestational diabetes (GDM) rates increase rapidly worldwide, along with increases in obesity, sedentary lifestyle and more vigilant screening. Intrauterine exposure to GDM and/or maternal overweight may be associated with increased cardiometabolic risk in the offspring. Screening and definition of GDM remain controversial and the long-term effects of intrauterine subthreshold hyperglycemia are unknown. We present the design of the

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FinnGeDi study, which is a population-based, prospective cohort study assessing the effects of GDM and maternal overweight on the future health of the mother and child.

The FinnGeDi study was launched in Finland in 2009 shortly after the introduction of new national GDM guidelines, changing GDM screening from risk factor based to universal. The study comprises three arms: 1. clinical case-control; 2. register-based; and 3. numerical oral glucose tolerance test (OGTT). Each arm serves as a base for follow-up of children and their mothers. In the clinical case-control arm, mothers with GDM and healthy controls, including their partners and babies (n=2000+2000 trios), are recruited from six antenatal clinics in Finland. Detailed phenotypic data plus DNA and plasma are collected. In the register-based arm Medical Birth Register data for all births in 2009 (n=59,921) are linked with extensive data from national registers for the newborn, parents and grandparents. In the third arm we collect numerical data of all OGTTs during pregnancy in six hospital districts.

Recruitment is expected to continue until 2013.

The homogeneous Finnish population, cost-free antenatal care and extensive register data through several generations make the FinnGeDi study unique. The clinical presentation of GDM is undergoing rapid changes along with Westernization and vigilant screening. The study will provide a contemporary perspective on risk factors of GDM and the short and long term consequences of GDM, maternal hyperglycemia and overweight for the health of the mother and child.

PII-140

Associations between Oxidative Stress in Pregnancy and Birth Anthropometry in a Cohort of Women and Children in Rural Bangladesh, the MINIMat-Trial. Emma Lindström[1], Samar Basu[2], Lars-Åke Persson[1], Rubhana Raqib[3], Shams El Arifeen[3], Eva-Charlotte Ekström[1]. *[1]Department of Women's and Children's Health, Uppsala, Sweden; [2]Department of Public Health and Caring Sciences, Uppsala, Sweden; [3]International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh.*

Oxidative stress is suggested as a mediating factor in the development of chronic metabolic diseases. We aim to investigate associations between oxidative stress in pregnancy and birth anthropometry.

In a community-based trial in Bangladesh, women were identified in early pregnancy and urine samples collected in week 14, 19, and 30. Oxidative stress markers were analyzed in a randomly selected subset. Free 8-iso-prostaglandin $F_{2\alpha}$(8-iso-$PGF_{2\alpha}$), a marker of lipid peroxidation, was analyzed in week 14 (n=374) and week 30 (n=308). 8-Hydroxy-2'-Deoxyguanosine (8-OHdG), a marker of DNA oxidation, was analyzed in week 19 (n=331).

Infant's weight, length, head and chest circumference was measured after birth. One-third were born low birth weight (LBW) defined as <2500 g. Categorical indicators for short length, small head and chest circumference was created by defining the lowest tertile as short and small respectively. Means of anthropometric indicators were analyzed with the GLM across oxidative stress tertiles. The odds for being in the smallest category of birth anthropometry in the different oxidative stress tertiles were analyzed using logistic regression adjusting for confounders.

Women in the highest 8-iso-$PGF_{2\alpha}$-tertile week 14 had infants with larger chest circumference ($mean_{adj}$ 31.4 ± 0.3 SE) compared to women in the lowest tertile ($mean_{adj}$ 30.6 ± 0.32 SE), $p < 0.05$. Women in the middle and highest 8-iso-$PGF_{2\alpha}$-tertiles also had lower odds of an infant with small chest (OR 0.51, 95% CI 0.29-0.91 and OR 0.48 95% CI 0.26.0.87) than women in the lowest tertile.

In analyses stratified for infant sex, women in the highest 8-iso-$PGF_{2\alpha}$-tertile week 14 had lower odds of boys born with small chest (OR 0.18, 95% CI 0.06-0.54) and of boys born LBW (OR 0.21, 95% CI 0.08-0.59) compared to women in the lowest tertile. In the strata of girls, higher 8-iso-$PGF_{2\alpha}$ in week 14 was also associated with lower odds of small chest but this was observed in the middle tertile.

In a population in Bangladesh were LBW is common, a higher level of oxidative stress in early pregnancy was associated with larger chest size at birth. In boys a lower odds of LBW was also a observed.

PII-141

Assocaition of CYP1A1, GSTs Genetic Polymorphisms and Passive Smoking with Preterm Delivery: A Case-Control Study in Southern China. Wei-Qing Chen[1], Yi-Juan Luo[1], Peng Ding[1], Yan-Hui He[1], Xiao-Lin Guo[2], Jian-Miao Lin[3], Tao Liu[1]. *[1]Department of Biostatistics and Epidemiology, Sun Yat-sen University, China; [2]Shengzhen Maternal and Child's Hospital, China; [3]Fushan Maternal and Child's Hospital, China.*

To investigate association between CYP1A1, GSTs genetic polymorphisms and passive smoking with preterm delivery

A case-control study was conducted on 198 women with preterm deliveries and 524 women with term deliveries in Southern China. Logistic regression anlaysis and standard delta method were used to study the association of maternal passive smoking and gene polymorphism in CYP1A1 and GSTs.

We found that maternal passive smoking was associated with an increased risk of PTD without consideration of genotypes (adjusted OR=2.20, CI:1.56-3.12). Compared with low risk genotype in non-passive smoking mother, CYP1A1 "AG/AA"+GSTs "present" genotype was synergistically interacted with maternal passive smoking (multiple interaction OR=2.92; CI:1.12-7.63, AP=0.69; CI:0.34-1.04), CYP1A1 "TC/TT"+GSTs "present" genotype was also found joint effects with maternal passive smoking exposure (AP=0.69; CI:0.34-1.04), the joint effects of passive smoking with GSTs genotype to PTD did not found.

The results of this study suggest that CYP1A1 "AG/AA" "TC/TT" mutation may increase the risk of PTD in passive smoking mother, both CYP1A1 and GSTs should be considered when assess the risk of CYP1A1 genotype associated with maternal passive smoking to PTD.

PII-142

Murine Brain Development Is Compromised by Gestational Exposure to Urban Air Pollution. Nilsa Regina Damaceno-Rodrigues[1], Karina do Valle Marques[1], Paulo Hilario Nascimento Saldiva[2], Elia Garcia Caldini[1], Mariana Matera Veras[2]. *[1]Laboratory of Cell Biology, Department of Pathology, University of Sao Paulo School of Medicine, Sao Paulo, Brazil; [2]Laboratory of Experimental Air Pollution, Department of Pathology, University of Sao Paulo School of Medicine, Sao Paulo, Brazil.*

The detrimental effects of air pollution on cardio respiratory health are well established. Besides these "usual" effects, some lesser-known effects loomed. Studies link gestational exposure to fine particulate matter (PM) to intrauterine growth restriction (IUGR) and more recently to damaging effects on brain growth and mental development. To test if brain development could be compromised by gestational exposure to fine PM we developed a murine model of real world exposure to air pollution.

Mice were maintained in exposure chambers located at a busy crossroads in São Paulo. They were raised and completed pregnancies in chambers with exclusively filtered (F) or non-filtered (NF) air. The 24-hr concentration of PM was determined gravimetrically. At 18-days gestation, dams were euthanized, and seven male fetuses per group selected (1/litter). Fetuses were weighed; the brain was dissected, weighted, fixed in formalin and processed for histological and stereological analysis.

Concentrations of PM in F chambers were significantly lower (76%, $p<0.001$) than those in NF chambers. Body weight was smaller in NF fetuses. The brain weight was significantly inferior in NF, but still proportional to the body weight. Stereological analysis of brain compartments revealed that NF fetuses presented significantly smaller cortex and cerebellum volumes; also brain ventricles (Lateral, III and IV) were less developed. When the volumes of brain compartments were analyzed in term of its contribution for total brain volume we observed that ventricular zone (VZ) was greater and hippocampus was lesser in NF fetuses.

Our results indicate that gestational exposure to air pollution could be associated to alterations of brain morphological development. Larger VZ, smaller ventricles and a less developed cortex suggest a delayed development of brain structures. Mechanisms involved in the impairment of fetal development are still unknown, but previous studies from our group (Veras *et al.* , *Biol Reprod* 79:578-84, 2008) indicate that air pollution exposure causes detrimental morphofunctional changes in the placenta, and therefore a compromised intrauterine environment could explain the observed effects.

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PII-143

Caffeine Intake during Pregnancy and Risk of Problem Behaviour at Age Five: Results from a Large Prospective Birth Cohort (ABCD-Study). Eva M. Loomans[1,3], Laura Hofland[2], Odin van der Stelt[1], Marcel F. van der Wal[3], Hans M. Koot[2], Bea R.H.M. Van den Bergh[1], Tanja G.M. Vrijkotte[4]. *[1]Department of Developmental and Clinical Psychology, Tilburg University, Netherlands; [2]Department of Developmental Psychology, VU University Amsterdam, Netherlands; [3]Department of Epidemiology, Documentation and Health Promotion, Public Health Service Amsterdam, Netherlands; [4]Department of Social Medicine, Academic Medical Centre, University of Amsterdam, Netherlands.*

Developmental programming of the foetus by intrauterine exposure to caffeine has been linked to adverse perinatal outcomes. The current study aimed to extend these findings and prospectively investigated the association between maternal caffeine intake during pregnancy and children's problem behaviour at age five.

In a large, multi-ethnic community based birth-cohort caffeine intake (coffee, caffeinated tea and cola) was measured (self-report) around the 16th week of gestation. Four groups with caffeine intake equivalent to 0-1, 2-3, 4-5, >5 cups of coffee/day were formed; I: 0 - 85 mg/day (reference), II: 86 - 255 mg/day, III: 256 – 425 mg/day and IV: > 425 mg/day. When children were five years old (M = 5.1, SD = 0.1), 4431 mothers and 3541 teachers have rated children's overall problem behaviour, emotional problems, conduct problems, hyperactivity/inattention problems and peer relationship problems with the Strengths and Difficulties Questionnaire (Goodman, 1997). Behaviour scores on all scales were dichotomized (potential problem behaviour: scores > 1 SD above the mean). When both mother and teacher ratings concurred children's behaviour was considered to be problematic. Multiple logistic regression analyses adjusted for the child's gender, maternal age, ethnicity, marital status, education, smoking, and alcohol consumption during pregnancy, family size and prenatal maternal anxiety were conducted. Mediation by foetal growth restriction and gestational age were tested. In addition, the moderating role of the child's gender was tested.

Caffeine intake was not associated with a higher risk for behaviour problems. No evidence was found for mediation by foetal growth restriction or gestational age. No moderation by the child's gender was found.

Results did not provide evidence for developmental programming influences of intrauterine exposure to caffeine on offspring's problem behaviour at age five.

PII-144

Chronic Low Dose Alcohol Consumption during Pregnancy Leads to a Down-Regulation of IGF1 in the Hearts of Rat Offspring. Vivian B. Nguyen[1], Monika Zimanyi[1], Karen M. Moritz[2], Megan E. Probyn[2], John F. Bertram[1], Mary J. Black[1]. *[1]Department of Anatomy and Developmental Biology, Monash University, Victoria, Australia; [2]School of Biomedical Sciences, University of Queensland, Queensland, Australia.*

Epidemiological studies demonstrate that individuals may be 'programmed' to develop cardiovascular disease as a result of insults that occur to the fetus in utero. Specifically, high levels of maternal alcohol consumption are known to affect cardiomyocyte development and maturation leading to permanent adverse structural changes in the heart with long-term deleterious effects in cardiac function. We aimed to determine whether low levels of alcohol consumption during pregnancy is also detrimental to cardiomyocyte growth and number in hearts of rat offspring.

Pregnant Sprague-Dawley rat dams were fed a control diet or 6% (volume/volume) liquid-based ethanol supplemented (isocaloric) diet throughout gestation. At embryonic day 20, expression of genes involved in cardiac development, maturation and apoptosis was analysed. Whilst at postnatal day 30, cardiomyocyte number in the left ventricle and adjoining septum was determined stereologically.

There was a significant (p=0.012) down-regulation of insulin-like growth factor 1 (IGF1) mRNA expression, but no differences were observed in IGF2, IGF1R or AT1bR levels which are also associated with cardiac growth. Similarly, apoptotic genes, such as Bax, Bcl-2 and p53 remained unaffected. However, the decrease in IGF1 gene expression levels did not appear to adversely affect heart growth, as no significant differences could be seen in heart weights or heart wall volume, or cardiomyocyte number in male or female offspring.

Overall, our results indicate that maternal consumption of alcohol at low levels during pregnancy is not detrimental to growth of the fetal heart. This suggests that effects of prenatal alcohol exposure on heart growth are dose dependent. This preliminary data is encouraging given the high proportion of pregnant women who chronically drink low quantities of alcohol throughout gestation.

PII-145

Periconception Maternal Smoking Is Associated with Birth Weight and Silencing of the Imprinted *INSIGF* Locus in the Child. Sylvia A. Obermann-borst[1], P. Eline Slagboom[3], Paul H.C. Eilers[2], Elmar W. Tobi[3], Eric A.P. Steegers[1], Bas T. Heijmans[3], Régine P.M. Steegers-Theunissen[1,4,5]. *[1]Obstetrics and Gynecology, Erasmus MC, Netherlands; [2]Biostatistics, Erasmus MC, Netherlands; [3]Molecular Epidemiology, LUMC, Netherlands; [4]Epidemiology, Erasmus MC, Netherlands; [5]Clinical Genetics, Erasmus MC, Netherlands.*

Maternal smoking is believed to be one of the major mediators between socioeconomic status and pregnancy outcome, the association between smoking and education status is apparent and can result in adverse birth outcomes such as SGA, low birth weight and IUGR. The underlying mechanisms, however, are not clear. We hypothesize that the methylation of *INSIGF*, an imprinted locus associated with SGA, is deranged due to periconception smoking and harmful exposures related to a low socioeconomic status. We aim to investigate associations between: 1) periconception maternal smoking, low educational level as proxy of exposures related to low socioeconomic status, and methylation of *INSIGF; 2)* the methylation of *INSIGF* and birth weight.

Of 120 children at 17 months of age genomic DNA was isolated from white blood cells and treated with bisulphite (Zymo Research). The methylation of 4 CpG dinucleotides in the promoter region of *INSIGF* was measured by mass spectrometry (Epityper, Sequenom). Periconception smoking was defined as smoking between four weeks prior until eight weeks after conception. Low education was defined by primary, lower vocational or intermediate secondary education. We applied Linear Mixed Model without imputation of missing values, adjusted for the correlation between individual CpG dinucleotides and bisulfite batch.

Of the mothers 32 (28.3%) smoked during the periconception period and 31 (25.8%) had a low education level. Periconception smoking and low education were independently associated with a relative increase in *INSIGF* methylation, +1.3%; p=0.043 and +1.6%; p=0.021, respectively. Smoking and low education showed a significant interaction on *INSIGF* methylation (+2.8%; p=0.011). Smoking was independently and inversely associated with birth weight (-231 gram; p=0.021), while education level was not associated with birth weight. The methylation of *INSIGF* CpG 5 was inversely associated with birth weight (P=0.010).

Our data suggest that the detrimental effect of periconception maternal smoking on birth weight could be explained in part by silencing of the imprinted *INSIGF* gene in the child.

PII-146

Infancy Growth and Menarcheal Age Are Associated with Anti-Müllerian Hormone, a Marker of Ovarian Reserve, in Early Adulthood. Jared M. Bragg, Monisha Banerjee, Thomas W. McDade, Christopher W. Kuzawa. *Northwestern University, IL, USA.*

Little is presently known about the role of developmental experiences in shaping female reproductive aging in humans. Epidemiological studies have suggested that small birth size and poor early postnatal growth predict earlier menopause, perhaps indicating a link between early growth conditions and initial follicular endowment. Additionally, as the rate of follicle loss is thought to slow at puberty, factors that influence timing of reproductive maturity could affect ovarian reserve indirectly. Here, we seek to clarify the role of developmental experiences in reproductive aging by assessing the relationship between birth weight, infancy growth, menarcheal age, and anti-müllerian hormone (AMH) levels, an indicator of ovarian reserve, in early adulthood.

AMH is secreted by growing ovarian follicles and is a non-invasive means of assessing ovarian reserve. Data are from young women (n = 271; age at blood draw = 21.5 yr) followed since birth in the Cebu Longitudinal Health and Nutrition Survey, conducted in Cebu City, the Philippines. Plasma AMH was

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measured by ELISA using a commercially-available kit. Multiple regression was used to model the effect of birthweight (adjusted for gestational age), weight velocity from birth to two years (in four, six-month increments), and menarcheal age on AMH in early adulthood, controlling for age and other factors known to be associated with AMH.
Greater weight velocity from 6-12 months and earlier menarcheal age were associated with higher AMH in early adulthood. The effect of 6-12 months weight velocity attenuates somewhat after adjusting for menarcheal age, suggesting that this effect is mediated in part by altering the time to maturation when the rate of follicle loss is thought to decrease. Birthweight was not associated with AMH.
Our finding that size at birth is not associated with AMH is in line with previous studies. To our knowledge, this is the first study to assess the relationship between early postnatal growth and AMH. These results suggest that the association between early menopause and poor infancy growth may be mediated by a direct effect on ovarian follicle number. The association between later menarcheal age and lower AMH could reflect a reduction of follicle number due to poor conditions during childhood growth or the altered timing of the post-pubertal hormonal milieu, which may have implications for patterns of follicle loss.

PII-147

Central Adiposity Is Associated with Sperm Quality in Men of Subfertile Couples. F. Hammiche, J. Laven, W. van Inzen, E. Steegers, R. Steegers-Theunissen. *Obstetrics and Gynaecology, Erasmus Medical Center Rotterdam, Netherlands.*
To study the effect of central adiposity and body mass index (BMI) on sperm parameters in men of subfertile couples.
In a preconception cohort study 455 men of subfertile couples were included from a preconception clinic 'Achieving a Healthy Pregnancy' in a tertiary outpatient fertility clinic. Main Outcome Measures were ejaculate volume (mL), sperm concentration (millions per mL), percentage of progressive motile and immotile spermatozoa and total motile sperm count (millions). Overweight was inversely associated with ejaculate volume (β-0.161(s.e.0.07); p=0.02) and the percentage of progressive motility (β-0.365(s.e.0.14); p=0.01), and positively associated with the percentage of immotile spermatozoa (β 0.210(s.e.0.06); p=0.001). Obesity was inversely associated with ejaculate volume (β-0.228(s.e.0.10); p=0.02), sperm concentration (β-0.678(s.e.0.26); p=0.009) and total motile sperm count (β -0.706(s.e.0.31); p=0.02). Waist circumference>102 cm, as measure for central adiposity, was inversely associated with sperm concentration (β-0.651(s.e.0.20); p=0.001) and total motile sperm count (β-0.621(s.e.0.24); p=0.01). All associations remained significant after adjustment for age, ethnicity, active and passive smoking, alcohol and medication use, folate status and andrological surgery.
This study shows that sperm quality in men of subfertile couples is significantly affected by BMI and in particularly central adiposity. The effect of weight loss on sperm quality and fertility needs further investigation.

PII-148

Defective Sertoli Cell Proliferation and Androgen Receptor Function in a Mouse Model of the *ATR-X* Syndrome. Stefan Bagheri-Fam[1], Anthony Argentaro[1], Terje Svingen[2], Alexander Combes[2], Andrew H. Sinclair[3], Peter Koopman[2], Vincent R. Harley[1]. *[1]Molecular Genetics & Development Division, Prince Henry's Institute of Medical Research, Victoria, Australia; [2]Division of Molecular Genetics and Development, Institute for Molecular Bioscience, The University of Queensland, Queensland, Australia; [3]Murdoch Childrens Research Institute and Department of Paediatrics, The University of Melbourne, Royal Childrens Hospital, Victoria, Australia.*
X-linked *ATR-X* (alpha thalassemia, mental retardation, X-linked) syndrome in males is characterized by mental retardation, facial dysmorphism, alpha thalassemia and urogenital abnormalities, including small testes. It is unclear how mutations in the chromatin remodeling protein ATRX cause these highly specific clinical features, since ATRX is widely expressed during organ development. We sought to investigate the mechanisms underlying the testicular defects observed in *ATR-X* syndrome.
We generated *ScAtrxKO* (Sertoli cell *Atrx* knockout) mice with Atrx specifically inactivated in the supporting cell lineage (Sertoli cells) of the mouse testis.
ScAtrxKO mice developed small testes (20% of control) and discontinuous tubules, due to prolonged G2/M phase and apoptosis of proliferating Sertoli cells during fetal life. We also found that the onset of spermatogenesis was delayed in postnatal mice, with a range of spermatogenesis defects evident in adult *ScAtrxKO* mice. ATRX and the androgen receptor (AR) physically interact in the testis and in the Sertoli cell line TM4 and co-operatively activate the promoter of Rhox5, an important direct AR target. We also demonstrate that ATRX directly binds to the *Rhox5* promoter in TM4 cells. Finally, gene expression of *Rhox5* and of another AR-dependent gene, *Spinlw1*, was reduced in *ScAtrxKO* testes.
These data suggest that ATRX can directly enhance the expression of androgen-dependent genes through physical interaction with AR. Recruitment of ATRX by DNA sequence-specific transcription factors could be a general mechanism by which ATRX achieves tissue-specific transcriptional regulation which could explain the highly specific clinical features of *ATR-X* syndrome when *ATR-X* is mutated.

PII-149

Inhibition of SRY-Calmodulin Complex Formation Induces Ectopic Expression of Ovarian Cell Markers in Developing XY Gonads. Helena Sim[1], Anthony Argentaro[1], Daniel P. Czech[1], Stefan Bagheri-Fam[1], Andrew H. Sinclair[2], Peter Koopman[3], Brigitte Boizet-Bonhoure[4], Francis Poulet[4], Vincent R. Harley[1]. *[1]Molecular Genetics and Development, Prince Henry's Institute of Medical Research, Victoria, Australia; [2]Murdoch Childrens Research Institute and Dept of Paediatrics, Royal Childrens Hospital, The University of Melbourne, Victoria, Australia; [3]Institute for Molecular Bioscience, The University of Queensland, Queensland, Australia.*
The transcription factor SRY plays a key role in human sex determination because mutations in SRY cause disorders of sex development in XY individuals. During gonadal development, Sry in pre-Sertoli cells activates Sox9 gene transcription, committing the fate of the bipotential gonad to become a testis rather than an ovary. The HMG domain of human SRY contains two independent nuclear localization signals (NLSs), one bound by calmodulin (CaM), and the other by importin-beta. While XY females carry SRY mutations in these NLSs which affect SRY nuclear import in transfected cells, it is not known if these transport mechanisms are essential for gonadal development and sex determination.
COS7 cells were transfected by Fugene 6 and CaM antagonists added 24h later. Luciferase activity was measured after 48h. For immunoprecipitation, cell extracts in RIPA buffer were subjected to IP with 2micrograms of CaM or Sry antibody. Immunohistochemistry was carried out by standard methods. Confocal laser scanning microscopy was used for quantitation of nuclear accumulation of Sry protein with NIH ImageJ. Mesogonads from Swiss mouse embryos (12-15 tail somites were cultured for four days with medium changes every 24h. Drug-supplemented culture medium was washed out by multiple changes with culture medium after 24h.
Here we show that mouse Sry protein binds CaM, and that a CaM antagonist reduces CaM binding, nuclear accumulation and transcriptional activity of Sry in transfected cells. CaM antagonist treatment of cultured, sexually indifferent XY mouse fetal gonads led to reduced expression of the Sry target gene Sox9, defects in testicular cord formation and ectopic expression of the ovarian markers Rspondin1 and Foxl2.
These results indicate the importance of CaM for SRY nuclear import, transcriptional activity, testis differentiation and sex determination.

PII-150

Early Life Determinants of Long-Term Reproductive Success in Swedish Males and Females Born 1915–1929. Anna Goodman[1,2], Ilona Koupil[2]. *[1]London School of Hygiene and Tropical Medicine, London, United Kingdom; [2]Stockholm University/Karolinska Institutet, Stockholm, Sweden.*
To study biological and social determinants of reproductive success in a post-demographic transition population.
We investigated early life determinants of mortality and fertility using multi-generational data from a large, population-based cohort of 13 666 individuals born in Sweden between 1915 and 1929. We studied the effects of birthweight for gestational age, preterm birth, birth multiplicity, birth order, mother's age, mother's marital status, family socio-economic

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position (SEP) and school performance at age 10. Our primary outcomes were probability of every marrying, total number of children and total number of grandchildren by end 2009.

By end 2009, 71% of our original cohort members had at least one child (69% males, 73% females) and 66% had at least one grandchild (64% males, 68% females). Males had a mean of 1.60 children and 2.94 grandchildren; females had a mean of 1.69 children and 3.19 grandchildren. The higher reproductive success of females resulted from survival advantages in child- and adulthood and also from a greater probability of marriage. In both sexes, higher birthweight for gestational age, full-term birth and a younger mother independently predicted a greater number of descendants. A married mother and higher family SEP also predicted a greater number of descendants in males (but not females), while in females (but not males) higher birth order predicted higher reproductive success. These effects were mediated by sex-specific effects upon the probability of marriage. In males (but not females), poorer school performance predicted fewer children and grandchildren, again primarily mediated via probability of marriage. The effect of school performance upon marriage in males was independent of early-life social and biological characteristics, and seemed to be largely mediated by adult SEP. Number of grandchildren increased with increasing number of children in both sexes, providing no evidence for a trade-off between quantity of offspring and their subsequent reproductive 'quality'.

Social and biological characteristics at birth and school performance at age 10 independently predict reproductive success in gender-specific patterns. The key mediating role of marriage provides a counterpoint to the lifecourse concept of 'embodiment', indicating how social characteristics may mediate the effects of early life biological and cognitive factors.

PII-151

Prophylactic Potential of Maternal Probiotic Supplementation Against Intestinal Dysfunctions Induced by Early Life Stress. Javad Barouei[1,2], Deborah Maree Hodgson[2]. *[1]Laboratory of Neuroimmunology, School of Psychology, The University of Newcastle, NSW, Australia; [2]Laboratory of Microbiology, School of Environmental and Life Sciences, The University of Newcastle, NSW, Australia.*

Both animal and human research has demonstrated that exposure to stress during the neonatal period of life results in alterations to the hypothalamic-pituitary-adrenal (HPA) axis resulting in offspring who hyper-respond to stress in adulthood. This is important given that stress has been reported to be an important causative or precipitative factor in Irritable Bowel Syndrome (IBS). All essential aspects of the brain-gut axis including HPA-axis, spinal pathways, immune system and the balance of enteric microbiota are altered by exposure to early life stress. This study aimed to determine whether maternal probiotic supplementation can act prophylactically to protect against intestinal dysfunctions induced by neonatal stress.

To test this, a probiotic combination of Bifidobacterium lactic Bb12 and Propionibacterium jensenii 702 was administered to pregnant and lactating Wistar rats via their drinking water. Control animals had free access to water without the probiotics added. After birth, pups were subjected to neonatal maternal separation (NS) for 3 h/day from postnatal day two to 14 or left undisturbed. At PND 24, pups were sacrificed and blood samples were collected to assess corticosterone levels. Small intestinal and colonic samples were collected for histochemistry. IIeal tissue samples were analysed for the level of mRNA expression of CRH receptor type 1 and 2, mucin (MUC), TNF-a and IFN-g. Faecal samples were analysed for the composition of gut microflora using RT-PCR.

Data analysis indicated that NS neonates demonstrated significantly higher plasma levels of corticosterone, higher ileal mRNA expression of CRH receptor type 1 and 2, TNF-a and IFN-g, lower mRNA expression of MUC and significantly higher faecal counts of Escherichia coli and significantly higher number of colonic mast cells compared with control pups. This situation was reversed to some extent when dams were treated with probiotics. Maternal probiotic use significantly decreased corticosterone level, ileal mRNA expression of CRH receptor, TNF-a and IFN-g, counts of E. coli and colonic mast cells and significantly increased MUC expression in NS neonate.

Our findings suggest that maternal probiotic intake ameliorates HPA axis and intestinal dysfunctions provoked by early life stress.

PII-152

Defining the Cellular Targets of IL6 in the Fetal Mouse Brain. Christine L. Jasoni, Shanti R.S. Campbell. *Centre for Neuroendocrinolgy, Department of Anatomy, University of Otago, New Zealand.*

Evidence from human and rodent studies indicates that maternal obesity results in elevated maternal and placental cytokines, including interleukin 6 (IL6). Our overarching hypothesis is that the programming effects of maternal obesity on offspring body weight come about through the actions of elevated maternal cytokines. As a first step toward addressing this hypothesis, we sought to define whether and which cells in the fetal brain are targets of IL6, and to discover whether there is a defined window during which the fetal brain is responsive to IL6.

Fetal brain sections were grown in organotypic slice culture for various times in the presence of three different concentrations of IL6. Following IL6 challenge, brain slices were fixed briefly in paraformaldehyde, and processed for dual-label immunohistochemistry for phosphorylated STAT3 (a marker of IL6 signaling activity) and a cell type-specific marker. The number of cells single labeled for pSTA3 were counted in different brain areas. In addition the number of cells double-labeled for pSTAT3 and a cell type-specific marker cells were also counted.

We have found that IL6 dose-dependently leads to an upregulation of pSTAT3 in multiple brain regions, including the hippocampus and hypothalamus. The specific cell types within each of these regions is currently being assessed. In addition, of the ages currently investigated (gestational day (GD) 15.5 and 17.5) there do not appear to be any differences in the anatomical distribution of responsive cells throughout the brain, although there is a trend toward reduced numbers of IL6-reponsive cells at GD17.5 when compared to GD15.5.

Our current findings indicate that multiple cell types in the fetal brain respond to IL6, and in so doing identifies the cellular targets of elevated maternal cytokines. Moreover, these data indicate that: 1) maternal/placental elevated IL6 could impact the development of numerous cell types within the fetal brain as a mechanism to bring about developmental programming of body weight; 2) there may be specific developmental windows during which time the fetal brain is susceptible to the programming effects of elevated maternal/placental IL6 exposure.

PII-153

Serial Polymicrobial Exposure Induces Immune Tolerance in Fetal Ovine Keratinocytes. Matthew W. Kemp[1], Masatoshi Saito[1,2], Jeffrey A. Keelan[1], Alan H Jobe[1,3], Shaofu Li[1], Tadashi Matsuda[2], Christine L. Knox[4], Boris W. Kramer[5], Jennifer G. Collins[5], John P. Newnham[1], Suhas G. Kallapur[1,3]. *[1]School of Women's and Infants' Health, The University of Western Australia, Western Australia, Australia; [2]Division of Perinatal Medicine, Tohoku University Hospital, Miyagi, Japan; [3]College of Medicine, University of Cincinnati, OH, USA; [4]Institute of Health and Biomedical Innovation, Queensland University of Technology, Queensland, Australia; [5]Department of Pediatrics, University Hospital, Maastricht, Netherlands.*

Intrauterine infection and inflammation is a major cause of preterm birth(1). In fetal sheep, repeated in utero exposure to E.coli lipopolysaccharide O55:B5 (LPS) causes immune paralysis(2,3). We aimed to compare the effect of single LPS exposure to sequential Ureaplasma parvum serovar 3(UP, a microorganism associated with preterm birth) and LPS exposure on the inflammatory response of preterm ovine epidermal keratinocytes (OEK).

OEK were isolated from preterm fetal skin (122 d) and cultured according to our established protocol (4). Identical OEK cultures were exposed to either i) 2 x 104 CFU UP for 24 h before being exposed to 1 ug of LPS for either 0.5, 1, 1.5, 2, 3 or 6 h or ii) 1 ug of LPS alone for 0.5, 1, 1.5, 2, 3 or 6 h4. IL-6, IL-8, and TNFα expression was assessed using quantitative PCR4. Each time point was performed in triplicate. Cells exposed to sterile UP media served as controls. One way ANOVA was used to assess differences in the mean transcript dCq value between groups.

As previously demonstrated, OEK exposed to LPS alone markedly increased expression of IL-6 ($p=0.05$), IL-8 ($p=0.008$), TNFα ($p=0.003$) relative to control (4). OEK serially exposed to UP and LPS increased expression of IL-6 ($p=0.023$), IL-8 ($p=<0.001$) and TNFα ($p=<0.001$) relative to control. In both groups, highest transcript expression was seen at 3 h post-LPS exposure. Relative to OEK exposed only to LPS, OEK exposed to UP and

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LPS revealed large reductions in IL-8 (231 v 2.5 p=0.011) and TNFα (164 v 7.5 p=0.010) expression (2^-ddcq) at 3 h post-LPS exposure. A large observed reduction in IL-6 expression (2^-ddcq) (13960 v 3.95 p=0.068) closely approached significance.
We present novel data suggesting immune paralysis of OEK following serial UP/LPS exposure. In utero exposure to multiple microorganisms is frequently associated with preterm birth and may contribute to skin infection in the preterm population via immune paralysis (5).

PII-154

Up-Regulation of Genes of Inflammation in the Placenta of IUGR in a Twin Model. Line Leduc[1], Maurice D. Bouity-Voubou[2], Alain T. Sane[2], Carole Garofalo[2], Edgard Delvin[2,3], Emile Levy[2]. *[1]Department of Obstetrics & Gynecology, CHU Sainte-Justine, QC, Canada; [2]Research Center, dept of nutrition, CHU Sainte-Justine, QC, Canada; [3]Research Center, dept of Biochemistry, CHU Sainte-Justine, QC, Canada.*
The placenta constitutes the active interface between the maternal and fetal environment.Placental insufficiency is one of the most significant process triggering IUGR, but the mechanisms involved remain uncertain. We propose that an inflammatory reaction is involved in this process. We sought to document in a twin model, with one co-twin being IUGR whether: 1) inflammation is activated and operational in the placenta by measuring protein expression of specific inflammatory biomarkers: circulating C-reactive protein (CRP), interleukins (IL-6 and IL 1ß) tumor necrosis factor - α and the nuclear factor of transcription NFκB 2) this inflammatory response was different in identical versus non indentical twins.
A specific protocol of sample mapping in the placenta was established. Three different sites for biopsies in each placenta were taken in nine monozygotic (MZ) and nine dizygotic (DZ) twins.Semi-quantitative RT-PCR were performed for the quantification of gene expression using 1.5 microliter cDNA from reverse transcription reaction as template. Total RNA was isolated with TRIzol and Primers were designed based on sequence homology between the mRNAs of human in order to determine the mRNA expression levels of inflammatory cytokines. The amount of proteins were measured by Western blots.
We observed that CRP transcripts and protein are increased in IUGR placenta($p<0.01$). Both mRNA coding for all the cytokines studied and their respective gene products were increased ($p<0.01$). These increases were observed irrespective of the localization from which the placental biopsies were obtained. We also documented by western immunoblotting, an increase in the transcription factor NF-κB with an unchanged concentration of IκB ($p<0.05$). No significant difference in intensity of the inflammatory process was observed between the MZ and the DZ twins.
An inflammatory process is actived and operational in the placenta. It is driven more by the immediate foetal environment than by the genetic background.

PII-155

Effect of Nucleoprotein Diet on Expression of Gene Candidates for Developmental Origins of Health and Disease (DOHaD). Tetsuo Ogawa[1,2], Randeep Rakwal[1,2], Junko Shibato[2], Chika Sawa[1], Tomomi Saito[1], Aya Murayama[1], Makiko Kuwagata[2], Haruaki Kageyama[2], Masaji Matsunaga[3], Kouji Usumi[4], Seiji Shioda[1,2]. *[1]Anti-aging Medicine Funded Research Labs, Showa University School of Medicine, Tokyo, Japan; [2]Department of Anatomy, Showa University School of Medicine, Tokyo, Japan; [3]Gene Trophology Research Institute, Japan; [4]Life Science Institute Co. Ltd., Japan.*
Epidemiological evidence has advanced a theory that undernutrition during gestation is an important early origin of adult diseases. Animal models have successfully demonstrated that maternal diet could contribute to some of the adult diseases. Undernutrition is harmful to fetuses whereas calorie restriction is beneficial in adults. Focusing on this opposite effect of nutritional condition, we previously reported a study on DNA microarray-based global gene expression profiling of maternal and fetal livers following food deprivation, using pregnant mice (C57BL/6J). The study identified 211 gene candidates responsible for DOHaD (Congenial Anomalies 2011, in press), including NADH dehydrogenase which can affect the longevity gene Sirt1 activity, and Dot1 (histone H3 methyltransferase). We were therefore interested to know whether some form of nutrition can reverse the changes in their expression. A diet rich in nucleic acids (NAs) and protamin, termed as nucleoprotein (NP), has been attracting a great deal of attention in food science for its beneficial effects. NP diet has been shown to improve learning and memory, prevent neuronal death, and relieve progression of rheumatoid arthritis in mouse models.
Here, we have conducted a global gene expression profiling in mice fed with NP diet rich in NAs from the salmon testis for four weeks. Total RNA extracted from the livers was pooled in each group (control and NP diet), prior to DNA microarray analysis (Agilent mouse whole genome 4 x 44K).
Results revealed nearly 1500 & 3500 up (>1.5 fold)- and down (<0.75 fold)-regulated genes following NP diet. Interestingly, among these genes, 53 genes were found to be common with the previously identified 211 candidate genes. To note, 19 out of the 53 genes were functionally unknown. Besides these, we also identified adenosine deaminase, IL-4, integrin alpha E, leukocyte-associated immunoglobulin-like receptor 1, and tachykinin four genes.
These results suggest that NP diet regulates the immune system that is one of the targets in DOHaD research. Possible roles of these genes will be further discussed.

PII-156

Perinatal Characteristics and Risk of Polio among Swedish Twins. Wei Perng[1], Sven Cnattingius[2], Anastasia Iliadou[3], Eduardo Villamor[1]. *[1]Dept. of Epidemiology, University of Michigan, School of Public Health, MI, USA; [2]Dept. of Medical Epidemiology and Biostatistics, Karolinska Institute and Hospital, Stockholm, Sweden; [3]Clinical Epidemiology Unit, Dept. of Medicine, Karolinska Institute and Hospital, Stockholm, Sweden.*
Prenatal exposure to adverse conditions has been related to increased adult mortality in world regions where infections are major causes of death. Consistent with this finding, low birth weight has been associated with higher risk of tuberculosis, a potentially deadly infection highly prevalent in many areas worldwide. It is uncertain whether perinatal conditions affect the risk of other severe infections throughout life. We examined the associations between indicators of intrauterine growth and risk of polio in later life.
Using prospectively collected data on 21,604 like-sexed Swedish twins of known zygosity born in 1928-1952, we examined the risk of polio in relation to birth length, weight, and head circumference (HC) using cohort and nested co-twin case-control analyses. Incidence of polio was determined through an interview conducted in 1998 and by cross-linkage with national inpatient and death registries using International Classification of Diseases codes versions 7-10.
There were 133 cases of polio. In the cohort analysis, birth length, weight, and HC were positively associated with risk of polio. After adjustment for sex, birth year, gestational age at birth, and within-twin pair correlations, twins of shortest length (44cm) had a statistically significant 67% (95% CI: 6%, 88%; p=0.04) lower risk of polio compared with the reference group (47-49cm). Similarly, twins in the lowest birth weight category (<2000g) had a 58% (95% CI: -1%, 83%) decreased risk of polio compared with those weighing 2000g-2500g. There was a positive trend between HC and risk of polio (P trend =0.05). In the co-twin control analyses among disease-discordant twins, birth length, weight, and HC were higher in cases than controls among both monozygotic (n=84) and dizygotic (n=142) twins. However, these associations were not statistically significant.
Lower birth length, weight, and HC were associated with decreased risk of polio later in life. Associations with length and HC suggest the effects might be related to early intrauterine growth restriction. Prenatal conditions may differentially affect susceptibility or response to infections depending on the timing of the insult and the type of immune response elicited by the agent.

PII-157

How Does Antenatal Endotoxin Affect on Postnatal Organ Growth in Rats? Effects of Dose and Sex. Keiji Suzuki[1], Hidehiro Takahashi[2], Hiroshi Masaki[3], Masanori Tamura[1]. *[1]Center for Maternal, Fetal and Neonatal Medicine, Saitama Medical Center, Saitama, Japan; [2]Department of Neonatology, Tokyo Metropolital Children's Hospital, Tokyo, Japan; [3]Department of Pediatrics, St Marianna University School of Medicine, Kanagawa, Japan.*

Chorioamnionitis is known to cause preterm delivery and impair development of various organs. The aim was to study effects of antenatal intra-amniotic lipopolysaccharide (LPS) on postnatal growth of various organs in rats.

At 20 d gestation, pregnant SD rats were anesthetized and the uterus exposed under general anesthesia. The uterine wall was punctured and 0.1 μg or 1 μg E. coli endotoxin, or saline (0.1mL) was injected into each amniotic sac (LPS 0.1, LPS 1 and saline group, respectively). In control pups (no tx group), no fetal surgery was performed. At 0, 1, 4 and 8 weeks after birth, the pups were euthanized, the brain, lung, heart, kidney and liver were collected and weighed.

The mortality rate was the highest in the LPS 1 group, followed by the LPS 0.1, saline and no tx groups. The body weights of LPS-exposed pups were lighter than saline or no tx pups at birth. At 8 wks, only in males, LPS 1 pups showed lighter body weight compared to pups in the other groups. Also only in LPS 1 males, the weight of the lung, heart, and liver were lower than those in the other groups. In contrast, the brain weight was not different and was proportional to the body weight in all groups.

Antenatal amniotic exposure to LPS had long-term influence on growth and development of various organs. Effects of fetal LPS exposure were different in different organs and influenced by LPS dosage and sex.

PII-158

Birth Size, Postnatal Growth and Mammographic Density in Mid-Life. Mary Beth Terry[1], Barbara A. Cohn[2], Ying Wei[1], Julie D. Flom[1], Lambert H. Lumey[1], Wenfei Zhang[1], Piera Cirillo[2], Stephen L. Buka[3], Ezra Susser[1], Catherine A. Schaefer[4], Karin B. Michels[5]. *[1]Epidemiology, Columbia, New York, NY, USA; [2]Public Health Institute, Berkeley, CA, USA; [3]Brown University, Providence, RI, USA; [4]Kaiser, Oakland, CA, USA; [5]Harvard School of Public Health, Boston, MA, USA.*

Larger birth size and faster rate of early infant and childhood growth have been associated with increased risk of some cancers later in life, particularly breast cancer. We conducted a prospective study of birth size and early life growth (measured by weight and height changes between birth and four years of age) and mammographic density in an adult follow-up of existing U.S. birth cohorts.

We followed women who were born from 1959 to 1967 and whose mothers participated in either the Collaborative Perinatal Project (Boston and Providence sites) or the Childhood Health and Development Study in California. Of the 1,134 women interviewed in adulthood (ranging in age from 39 to 49 years at interview), 73% had a screening mammogram. We measured density using the Cumulus computer thresholding program.

After adjusting for maternal pre-pregnant body mass index and maternal education, larger changes in weight from birth to four months and from one to four years were statistically significantly associated with lower mammographic density (β=-1.5, 95% Confidence Interval (CI) = -2.3, -0.7 per 10% change in percentile weight gain from birth to four months; β=-1.6, 95% Confidence Interval (CI) = -2.5, -0.7 per 10% change in percentile weight gain from one to four years). Birthweight and weight changes from four months to one year were not associated with mammographic density after considering weight changes in the other time periods. Birth length and height changes were not associated with mammographic density. These effects were partially, but not fully, mediated by adult body size and menopausal status (β=-0.8, 95% Confidence Interval (CI) = -1.5, -0.1 per 10% change in percentile weight gain from birth to four months; β=-1.0, 95% Confidence Interval (CI) = -1.9, -0.2 per 10% change in percentile weight gain from one to four years).

Given the strength of mammographic density as an intermediate marker for breast cancer, the inverse associations between early weight changes and mammographic density suggest that the overall positive association between larger birth and infant size and breast cancer are not explained by mammographic density.

PII-159

Results from an RCT Pilot Study of Prenatal Interventions to Reduce Maternal Stress and Excessive Weight Gain — Assessing Effects on Offspring Physiology, Adiposity, and Growth. Nicole Bush, Nancy Adler, Elissa Epel, Barbara Laraia. *University of California, San Francisco, USA.*

Although there is clear evidence for effects of prenatal maternal stress and weight gain on offspring physiology and anthropometry, there are almost no studies of interventions during pregnancy to ameliorate those risks, and even fewer with randomized control designs. We will present initial results from the pilot phase of a U-01 funded RCT of two prenatal wellness interventions designed to improve the prenatal programming effects of maternal stress and eating during pregnancy on offspring physiology, growth, and development.

The enrolled pilot sample consists of 24 ethnically diverse, overweight (BMI 25-40) pregnant women from low- to middle-income families in San Francisco, who are undergoing one of two 8-week intervention conditions. Maternal prenatal assessments include comprehensive measures of current subjective stress, biological markers of stress (e.g. CRH, salivary and hair cortisol) and metabolism (glucose, insulin, leptin), and state-of the art measures of diet (dietary recall) and weight and adiposity gain (hip/waist circumf., Bod-Pod, ultrasound of abdominal and visceral adiposity). Infant data will include delivery outcomes, neurobehavioral outcomes (APGAR scores, child temperament, neuromotor functioning), physiologic stress reactivity (e.g. HPA reactivity to stressor), and body composition (weight-for-length and adiposity at birth and 1-month).

Maternal prenatal data is collected. Births are expected Jun-Aug of 2011. Birth records, as well as 1-month infant postnatal assessments will be collected in time for the conference. Linear regressions will test for associations between predictors and infant outcomes, and ANOVAs will test for intervention differences.

The random assignment of maternal participants to condition is a rare opportunity to assess the effects of reduction in prenatal stress and improvements in maternal weight control on a set of key offspring physical, behavioral and biological outcomes that are directly relevant to future disease risk. Pilot results will inform design of the full phase III RCT (N=180) to begin October 2011. Both the pilot and full project will identify modifiable gestational antecedents of children's maladaptive physiologic and metabolic responses that are early drivers of disease risk. Initial findings will be discussed and input from the DOHAD community will be sought on the phase III project.

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Withdrawn by Author

PII-161

Maternal Exercise during Pregnancy Improves Glucose Disposal in Mice Offspring. Lindsay G. Carter[1], Kaitlyn N. Lewis[2,3], Preetha Shridas[4], Mary L. Garcia-Cazarin[5], Francisco H. Andrade[5], Rafael de Cabo[2], Kevin J. Pearson[1]. *[1]Graduate Center for Nutritional Sciences, University of Kentucky College of Medicine, KY, USA; [2]Laboratory of Experimental Gerontology, National Institute on Aging, National Institutes of Health, MD, USA; [3]Departments of Cellular & Structural Biology and Physiology, University of Texas Health Science Center, TX, USA; [4]Department of Internal Medicine, University of Kentucky College of Medicine, KY, USA; [5]Department of Physiology, University of Kentucky College of Medicine, KY, USA.*

Although exercise is widely recognized as an important part of a healthy lifestyle and is known to improve cardiovascular and metabolic health, seemingly few people have the time or motivation for physical activity. However, if short-term exercise, particularly during pregnancy, not only protects individuals against disease but provides lifelong health benefits to the developing child, individuals may be more motivated to do so. We hypothesized that maternal exercise during pregnancy will improve glucose disposal in offspring by increasing peripheral insulin sensitivity, possibly leading to a reduction in diabetes risk over a lifetime.

Female ICR mice were separated into sedentary or exercise cohorts, and the exercise females were given voluntary access to a running wheel in their home cage prior to and during pregnancy as well as nursing. Offspring were

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weaned and analyses were performed on the mature offspring that did not have access to running wheels during any portion of their adult lives. To evaluate glucose regulation, glucose tolerance tests were performed in male and female offspring (n = 18 – 20 per group per sex). Insulin sensitivity was measured by insulin tolerance test as well as ex vivo analysis of 2-deoxyglucose uptake in muscle and adipose in response to insulin.
Maternal exercise was found to significantly improve glucose homeostasis in male and female offspring born to exercised dams through enhanced insulin sensitivity in both muscle and adipose.
These findings are an exciting first step in elucidating the long-term potential benefits of maternal exercise on offspring health. Furthermore, if these findings are conserved in humans, maternal exercise could be an excellent, short-term intervention to target cardiovascular disease and diabetes in the next generation.

PII-162

Pregnancy Outcome Experience in Ongoing Food Based Micro-Nutrient Supplementation Trial. Ramesh Potdar[1], Meera Gandhi[1], Sujay Joshi[1], Caroline Fall[2]. *[1]Centre for the Study of Social Change, Mumbai, India; [2]Medical Research Council, University of Southampton, United Kingdom.*
Mumbai Maternal Nutrition Project is ongoing trial of food based micronutrient supplementation in slum women before and during pregnancy with the primary outcome of increase of birth weight of newborns by 100 gms .Micronutrients are delivered in the form of Green Leafy Vegetables, Milk and Fruit constituted in a recipe to be eaten under observation by registered women at least three times a week. Trial, in an advanced stage now, is not complete yet and no interim or final results can be available. Albeit, we can learn a lot through process analysis of 1057 babies born in this trial during last four years. There are eight points of sequential contacts for every infant from birth to five years when Anthropometry and Developmental assessment using DASII (Developmental Assessment Scale for Indian Infants) scale was done on each child. Tabular results will be presented at length in the main presentation.
Birth Weight, Length and Infant Anthropometry is done for every infant from birth to five year of age by trained project staff. DASII (Developmental Assessment Scale for Indian Infants) scale is used to measure the developmental assessment at the age of six months and one year by trained project staff.
Lessons to be learnt could be summarized as follows:
• 15% deliveries (pregnancy outcomes) cannot be measured at birth because of women going for delivery to their native villages.
• Nearly 15% newborns are likely to miss first visit at 1month of age due to going to native place after delivery.
• 15% babies are lost to follow up at the end of three years due to reasons such as long stay at village, permanent shift of residence, parental refusal due to “evil eye” and/or sickness.
• Developmental Assessment; 624/939 (66%) at the end of six months; 653/830 (78%) at the end of one year
• Incidence of Low Birth weight (<2.5 kg) was 83/1057 (7.8%)
• Gestation >42 wks: 32/1057(7.8%)
• Gestation <37wks: 217/1057(20.5%)
This information stress that consideration needs to be given to possibility of dropouts and constraints in advance before calculating sample size for new trials.

PII-163

Effect of Fish Oil Supplementation during Pregnancy on Risk Factors for the Metabolic Syndrome in the 19 Year Old Offspring. Dorte Rytter[1], Bodil H. Bech[1], Jeppe H. Christensen[2], Erik B. Schmidt[3], Tine B. Henriksen[4], Sjurdur F. Olsen[5]. *[1]Department of Epidemiology, School of Public Health, Aarhus University, Denmark; [2]Department of Nephrology, Aalborg Hospital, Center for Cardiovascular Disease, Aarhus University Hospital, Denmark; [3]Department of Cardiology, Aalborg Hospital, Center for Cardiovascular Disease, Aarhus University Hospital, Denmark; [4]Department of Pediatrics, Skejby Hospital, Aarhus University Hospital, Denmark; [5]Centre for Fetal Programming, Statens Serum Institut, Denmark.*
Supplementation with long chained n-3 polyunsaturated fatty acids (PUFA) in adulthood has been found to be protective against several risk factors involved in the metabolic syndrome. It is, however, not known whether n-3 PUFA supplementation during pregnancy also can affect the development of the various components of the metabolic syndrome in the offspring.
The aim of the present study was to investigate the effect of supplementation with 2.7 grams of long chain n-3 PUFA (fish oil) during pregnancy on risk factors associated with the metabolic syndrome in the 19 year old offspring.
The study was based on a long term follow-up of a randomized controlled trial from 1990, in which 533 pregnant women were randomized to fish-oil (n=266), olive-oil (n=136) or no oil (n=131). In 2009, the offspring were invited to a physical examination where anthropometric measures, blood pressure and heart rate variability (HRV) were measured. Also a fasting venous blood sample was drawn and analyzed for lipids, lipoproteins and apolipoproteins, insulin, glucose, HbA1c, leptin, adiponectin, IGF-1 and hsCRP concentrations. Multiple linear regression modeling, adjusting for relevant confounders was used to estimate the effect of fish oil relative to olive oil on the different outcomes.
243 of the offspring were followed up. There was no difference in BMI ((0.13 (-0.92; 1.17) kg/m^2), waist circumference (0.7 (-2.1; 3.4) cm), systolic blood pressure (1 (-2; 4) mmHg) and diastolic blood pressure (1 (-1; 3.) mmHg) between the FO and OO groups. Also, no difference was found for heart rate and HRV. Overall, no effect of fish oil supplementation was found on the biochemical parameters.
We could detect no effect of fish-oil supplementation during pregnancy on any of the risk factors associated with the metabolic syndrome in the offspring.
Some of the results here have been presented in a preliminary form at CELSE 2010, Cyprus and at the Power of Programming, Münich 2010.

PII-164

Bone Marrow Stem Cell Therapy in Hypertensive Disorders of Pregnancy Improves Placental Circulation, Maternal and Fetal Outcome. Sudha Y. Sane[1], Satish Patki[2]. *[1]SNEHA India, India; [2]Patki Hospital & Research Center, Maharashtra, India.*
To observe the effects of bone marrow stem cell therapy 1)on improvement in maternal disease & fetal outcome 2)on placental ischemia & villous vessels.
We registered 31 cases beyond 20 weeks gestation. Nineteen of these formed study group for MNC therapy that included A)Preeclampsia 12 cases B) Gestational Hypertension (without proteinuria) three cases C)Chronic hypertension with pregnancy four cases D)Control group constituted three cases of conventionally treated preeclampsia and nine cases of normal gestation. Cases with symptoms of impending eclampsia or coexistent other major disease were excluded.
MNC therapy was given during 20 to 24 weeks gestation. Approximately 100ml of bone marrow was obtained from iliac crest. MNC were separated washed and about 80x10^6 cells re-suspended in 2ml of patients own serum. This was transplanted on placental base near center under ultra-sound guidance.
Pre and post treatment maternal parameters included blood pressure, proteinuria, obstetric sonography to assess fetal growth and amniotic fluid index. Gestation period at delivery and baby’s weight were recorded.
Placental weight and infarcts were recorded. Microscopically using immuno-stains CD 34 for endothelium, number of capillaries per villous were counted with the help of computer based soft ware (Image drafter).
All cases of study group responded within one week of MNC treatment with excellent response in preeclampsia and less in chronic hypertension. Systolic and diastolic B.P. dropped significantly (P= 0.0003), AFI increased and fetal growth improved. Sixteen of the nineteen cases in study group delivered at full term with baby’s weight above 2.5kg. Three cases delivered pre-term with babies weighing 1.2-1.5kg. Average birth weight for normal controls was 2.5-3.2kg and preeclampsia control was 1.2-1.5kg. Average weight of placenta in study group was 414±97gm in normal control was 556± 45gm and in preeclampsia control 370gm. Many infarcts were seen in preeclampsia control, few in normal control and none in study group. Capillary average co-related with placental weight in all cases (r=0.478, p=0.016). Capillaries per villous were significantly higher in MNC treated study group and positively co-related to birth weight (r=0.768 p=0.001)
MNC treatment significantly improved maternal condition, fetal growth and placental circulation. 16 out of 19 cases of study group delivered full term with normal birth weight.

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Community Practitioners' Experiences of a Public Health Intervention: Barriers and Facilitators to Skill Implementation. Tannaze Tinati[1,2], Wendy Lawrence[2], Georgia Ntani[2], Christina Black[1,2], Sue Cradock[1], Megan Jarman[1,2], Anna Pease[1,2], Hazel Inskip[2], Cyrus Cooper[2], Janis Baird[2], Mary Barker[2]. *[1]NIHR Biomedical Research Unit Nutrition, Diet and Lifestyle, Southampton University Hospitals Trust, United Kingdom; [2]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom.*

Improving health care professionals' communication skills to facilitate behaviour change was a key recommendation in public health guidance from the National Institute of Health and Clinical Excellence, UK. An intervention to improve maternal and child nutrition involved training community practitioners in 'Healthy Conversation' skills: techniques intended to help them better support mothers in making changes to their diets and physical activity levels. This study aimed to explore barriers and facilitators to using these skills and to examine the relationship between barriers and frequency of skill use.

Barriers and facilitators to using the skills were assessed with an adapted version of the Problematic Experiences of Therapy scale (PETS). One hundred and one community practitioners, who attended one of 13 workshops following the training, completed an adapted PETS and short questionnaire asking how often they were using the new skills. They were also asked to describe what made it more difficult or easier to use the skills.

Results indicated that staff had confidence in their use of the skills, but experienced some practical barriers such as identifying opportunities to use them. The fewer the barriers the staff perceived, the more often they implemented their new skills. Skills were used less often when staff perceived mothers to respond less well to their use (r_s=-0.42,p<.001), when staff felt less confident to use the skills (r_s=-0.37,p<.001), and when there were more practical problems (r_s=-0.37,p<.001).

Qualitative analysis of the free-text responses suggested that the main barriers related to staff's perceived lack of time to have healthy conversations in their everyday work. Facilitators included finding appropriate opportunities, positive experiences of using the skills and receptive mothers. Understanding these barriers and facilitators enables the training team to further support staff to embed their new skills in practice, and hence be more effective in empowering mothers to improve their diets and increase their physical activity levels.

PII-166

Non-Steroidal Anti-Inflammatory Drug Treatment: Effects on the Developing Preterm Baboon Kidney. Megan R. Sutherland[1], Bradley A. Yoder[2], Donald McCurnin[3], Steven Seidner[3], Ronald I. Clyman[4], Mary J. Black[1]. *[1]Anatomy and Developmental Biology, Monash University, Victoria, Australia; [2]Department of Pediatrics, University of Utah, UT, USA; [3]Department of Pediatrics, University of Texas Health Science Center, TX, USA; [4]Department of Pediatrics, University of California, CA, USA.*

Nephrogenesis (the development of nephrons in the kidney) occurs postnatally following extremely preterm birth (birth prior to 28 weeks gestation). In the neonatal period, preterm infants are commonly administered medications for treatment of patent ductus arteriosus, including non-steroidal anti-inflammatory drugs (NSAIDs) and nitric oxide sythase inhibitors (NOSi). Morphologically abnormal glomeruli, with an enlarged Bowman's space and shrunken glomerular tuft, are commonly observed in the outer renal cortex of the preterm kidney. The cause of these abnormalities is unknown. The aim of this study was to determine whether early postnatal non-steroidal anti-inflammatory drug treatment is the cause of the glomerular abnormalities observed in the preterm kidney.

Baboon neonates were delivered prematurely at 125d gestation (term = 185d) and were euthanized at birth or at postnatal day 6. Neonates were divided into four groups: 125d gestational controls (n=4), Untreated (n=8), Ibuprofen (n=6), and Ibuprofen+NOSi (n=3). Animals in the Ibuprofen and Ibuprofen+NOSi groups received five doses of ibuprofen, at 24 hr intervals, with the Ibuprofen+NOSi animals additionally administered a nitric oxide synthase (NOS) inhibitor (L-NMMA).

There was no difference between groups in body weight, kidney weight or glomerular generation number. Nephrogenic zone width was significantly reduced in the Ibuprofen group compared to the 125d gestational control and Untreated animals. Morphologically abnormal glomeruli were present at a range of 0.0% to 22.9% in the Untreated group, 0.0% to 6.1% in the Ibuprofen group, and 0.0% to 1.4% in the Ibuprofen+NOSi group.

Early postnatal ibuprofen exposure is associated with a reduced nephrogenic zone width; however, it is not the cause of the abnormal glomerular morphology associated with preterm birth.

PII-167

Undernutrition Fetal Programming: Effects on Kidney Morphology, Renal Steroid and Angiotensin Receptors Expression and Urinary Sodium Excretion. Bárbara Vaccari[1], Flávia F. Mesquita[2], José A.R. Gontijo[2], Patrícia A. Boer[1]. *[1]Department of Morphology, São Paulo State University, São Paulo/Botucatu, Brazil; [2]Department of Internal Medicine, State University of Campinas, São Paulo/Campinas, Brazil.*

The present study investigates, in adult male rats, the effect of food restriction *in utero* on arterial blood pressure changes (AP), and its possible association with the number of nephrons, renal function and angiotensin II ($AT1_R$/$AT2_R$) and glucocorticoid (GR) receptors expression.

The daily food supply to pregnant rats was measured and one group (n=5) received normal quantity of food (NF) while the other group received 50% of that (FR50) (n=5). The AP was measured weekly. Fractionator's method was used to estimate glomeruli number in histological slices. Kidneys were also processed to AT1, AT2 and GR immunolocalization and for western blotting analysis. The renal function was estimate by creatinine and lithium clearances Blood and urine samples were collected to biochemical determination of creatinine, sodium, potassium and lithium.

FR50 offspring shows a significant reduction in BW (FR59: 5.67 ± 0.16 *vs.* 6.84 ± 0.13g in NF, p<0.001) and increased AP from 6th to 12nd week (6thwk FR50: 149.1 ± 3.4 *vs.* 125.1 ± 3.2mmHg in NF, p<0.001and, 12ndwk FR50: 164.4 ± 4.9 *vs.* 144.0 ± 3.3 mmHg in NF, p=0.02). By stereological analyses, FR50 offspring present a reduction of nephron numbers per kidney (47%, p=0.007) with unchanged renal volume when compared with NF group. Expression of AT1 and AT2 were significantly decreased in FR50 (AT1, 59080 ± 2709 *vs.* 77000 ± 3591 in NF, p=0.05; AT2, 27500 ± 95.50 *vs.* 67870 ± 1509 in NF, p=0.001) while the expression of GR increased in FR50 (36090 ± 781.5 *vs.* 4446 ± 364.5 in NF, p=0.0007).We also verified a pronounced decrease in fractional urinary sodium excretion in FR50 offspring (0.03±0.02 *vs*. 0.06±0.04 in NF, p=0.03). This occurred despite unchanged creatinine clearance.

The study led us to suggest that fetal undernutrition, with increased fetal exposure to maternal corticosteroids, *program* to persistent renal glucocorticoid receptor upregulation in adulthood life. That effect may be related to development of hypertension in progenies. Additionally, the reduction in nephron number and downregulation of the angiotensin II receptors may result in lack of ability of renal tubules water and salt handling, which in turn may also contribute to hypertension establishment.

FAPESP supported this work.

PII-168

IUGR-Induced Long Term Hypertension and Chronic Kidney Disease, in the Rat, Depends on "Nephron Number Dosing". Farid Boubred[1,2], Laurent Daniel[3], Christophe Buffat[4], Charles Oliver[4], Michel Tsimaratos[4], Martine Lelievre-Pegorier[5], Françoise Dignat-George[1], Umberto Simeoni[1,2]. *[1]INSERM UMR608, Faculté de Pharmacie, Université de la Méditerranée, Marseille, France; [2]Division of Neonatology, Hôpital la Conception, Assistance Publique - Hôpitaux de Marseille, Marseille, France; [3]UPRES EA3281, Faculté de Médecine, Université de la Méditerranée, Marseille, France; [4]UPRES EA2193, Faculté de Médecine, Université de la Méditerranée, Marseille, France; [5]INSERM U652, Institut Biomédical des Cordeliers, Marseille, France.*

Low birth weight is a risk factor of hypertension (HT) and growing evidence suggest its role on chronic kidney disease (CKD). Nephron number deficit associated with intrauterine growth restriction (IUGR) is postulated to induce HT and CKD. However, the role of such a mechanism is still discussed.

The purpose of our study was to evaluate, through two different IUGR models how long term HT and CKD vary according the experimental model and how "nephron nephron dosing" plays a determinant role.

IUGR was obtained from two different models: maternal protein diet restriction (MLP, casein 9%) and gestational administration of betamethasone (BET, 0.25 mg/kg BW three days, E17-E19). Blood pressure

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(BP, tail cuff plethysmography), glomerular filtration rate (GFR, clearance of creatinine,ClCreat) were measured periodically until mo 15; and renal structure (glomerular counting and glomerular sclerosis) was evaluated in 22 mo-old offspring.
Both types of prenatal intervention led to IUGR, with a similar degree: birth weights were 17% and 20% lower in MLP and BET offspring as compared with control ($p < 0.01$). BET offspring had severe nephron number deficit (-50%, -40% in male and female offspring vs control), impaired GFR (ClCreat was 33% lower than for control) before the occurrence of hypertension (BP, + 20 mmHg , + 17 mmHg in males and female offspring vs control), and significant glomerulosclerosis (3.5 fold ($p < 0.001$) and two fold ($p < 0.05$) higher in male and female BET ageing offspring compared to both control and MLP offspring).Long term BP, GFR and renal structure.
While differences exist regarding the initial conditions leading to IUGR, these findings suggest (i) that birth weight is one but not the only predicting factor of nephron number endowment and long term adult diseases, and (ii) that "nephron number dosing" plays an important role in the "fetally programmed" adult vascular and renal diseases.

PII-169

Human Glomerular Number and Size: Correlations with Birth Weight. Rebecca N. Douglas-Denton[1], Monika A. Zimanyi[1], Michael D. Hughson[2], Wendy E. Hoy[3], Susan A. Mott[3], Libby Holden[3], Victor G. Puelles[1], John F. Bertram[1]. *[1]Anatomy and Developmental Biology, Monash University, VIC, Australia; [2]Pathology, University of Mississippi Medical Center, MS, USA; [3]Centre for Chronic Diseases, University of Queensland, Queensland, Australia.*
Human nephrogenesis finishes at approximately 36 weeks gestation, after which no new nephrons form. Therefore, perturbations to nephrogenesis result in a permanent nephron deficit and may influence mean and variability of glomerular size. Our aim was to define associations between birth weight, total glomerular number (N_{glom}), mean glomerular volume (V_{glom}) and individual glomerular volume (IGV).
Kidneys were obtained from Caucasian and African Americans at autopsy in Jackson, Mississippi, USA. Tissue samples were processed for embedding in glycolmethacrylate and unbiased stereological methods were used to estimate N_{glom}, V_{glom} and IGV. For IGV estimation, 12 Caucasian and 12 African Americans were selected and carefully age-matched based on their birth weight (6 with the lowest and highest birth weights per race).
N_{glom} varied widely in both racial groups: African Americans (n = 184; mean 898,821; range 210,332 – 2,702,079), Caucasian Americans (n = 141; mean 912,446; range 227,327 – 1,901,542). In both groups, there was a strong direct correlation between birth weight and N_{glom} (African Americans n = 127, $r^2 = 0.15$, $p<0.0001$; white Americans n = 75, $r^2 = 0.16$, $P = 0.0003$). In 15 children younger than three months, N_{glom} ranged 4.5-fold, suggesting most of the variation in adult N_{glom} is present at birth. V_{glom} was inversely associated with N_{glom} in adults of both racial groups (African Americans n = 156, $r^2 = 0.16$, $p<0.0001$; white Americans n = 131, $r^2 = 0.24$, $p<0.0001$). In African Americans, larger mean IGV (SD) was found in the low birth weight group (5.75 (1.49) vs 4.40 (1.14) x 10^6 μm^3; $p=0.049$) and in Caucasian Americans we found a similar trend (5.24 (1.17) vs 3.72 (1.06) x 10^6 μm^3; $p=0.045$). Pooled data was highly significant ($P<0.0001$) after adjustment for age and body size. IGV variation within subjects ranged from 2.0 to 11.2-fold in African Americans and from 1.7 to 6.5-fold in Caucasians.
These findings demonstrate a wide variation in human adult N_{glom}, with values ranging at least 13-fold. V_{glom} also varies widely between subjects, and is powerfully regulated by N_{glom}. IGV within single kidneys also varies widely, and this heterogeneity is influenced both by N_{glom} and birth weight.

PII-170

Perinatal Exogenous Nitric Oxide in Fawn-Hooded Hypertensive Rats That Persistently Lowers Blood Pressure in Adult Life Reduces Renal Ribosomal Biogenesis in Early Life. Sebastiaan A. Wesseling[1], Paul B. Essers[2], Maarten P. Koeners[1], Tamara C. Pereboom[2], Branko A. Braam[3], Alyson W. MacInnes[2], Jaap A. Joles[1]. *[1]Nephrology & Hypertension, University Medical Center Utrecht, Netherlands; [2]Hubrecht Institute Utrecht, Netherlands; [3]Nephrology and Immunology/Dept Medicine and Dept of Physiology, University of Alberta, Edmonton, AB, Canada.*
Nitric oxide (NO) is known to depress ribosome biogenesis in vitro. In this study we analyzed the influence of exogenous NO on ribosome biogenesis in vivo using a proven antihypertensive model of perinatal NO administration in genetically hypertensive rats.
Fawn-hooded hypertensive rat (FHH) dams were supplied with the NO donor molsidomine in drinking water from two weeks before to four weeks after birth, and the kidneys were subsequently collected from two day, two week and 9-10 month old adult offspring.
Microarray analysis revealed marked differential up-regulation of ribosomal protein genes at two days and down-regulation at two weeks and in adult males. Such differential regulation of ribosomal protein genes was not observed in females. These changes were confirmed in males at two weeks by expression analysis of ribosomal protein L36a and by polysome profiling, which also revealed a down-regulation of ribosomes in females at that age. However, polysome profiles returned to normal in adults after early exposure to molsidomine. No direct effects of molsidomine were observed on cellular proliferation in kidneys at any age, and the changes induced by molsidomine in renal polysome profiles at two weeks were absent in the livers of the same rats.
Our results suggest that the prolonged antihypertensive effects of perinatal NO administration may be due to alterations in renal ribosome biogenesis during a critical period of renal development, and provide a salient example of a drug-induced reduction of ribosome biogenesis that is accompanied by a beneficial long-term health effect.

PII-171

Renal Dysfunction Is Associated with Reduced Nitric Oxide Production in Sheep Following Fetal Uni-Nephrectomy. Yugeesh R. Lankadeva[1], Reetu R. Singh[2], Andrew J. Jefferies[2], Kate M. Denton[1], Karen M. Moritz[3]. *[1]Physiology, Monash University, Victoria, Australia; [2]Anatomy and Developmental Biology, Monash University, Victoria, Australia; [3]School of Biomedical Sciences, University of Queensland, Brisbane, Australia.*
An adverse *in utero* environment is associated with cardiovascular and renal disease in adulthood. A common finding in developmental programming models is a reduction in nephron endowment. Our model directly examines the consequences of a congenital nephron deficit in programming hypertension, without the confounding effects of reduced birth weight often linked with other models. We hypothesized that the control of renal vascular function would be altered in the uni-x animals, such that the response to nitric oxide (NO) inhibition would be blunted.
In our sheep model, fetal unilateral nephrectomy (uni-x) is performed at 100 days of gestation (period of rapid nephrogenesis, term = 150 days). In the current study, we examined mean arterial pressure (MAP) and renal function (Glomerular filtration rate; GFR, via clearance techniques) in a cohort of female sheep at four years of age. In conscious female uni-x (n=8) and sham (n=8) sheep, MAP and GFR, were measured before and during L-NAME (bolus 40mg/kg plus 20mg/kg/h i.v.) infusion.
Basal MAP was elevated in the uni-x sheep at four years of age compared to sham animals (96.8 ± 1.5 vs 80.2 ± 3.2 mmHg respectively; P <0.001), whilst basal GFR was significantly reduced (0.67 ± 0.06 vs 0.98 ± 0.07 ml/min/gkw respectively, P<0.001). In response to L-NAME infusion, MAP increased by ~14 mmHg in both uni-x and sham animals ($P_{Treatment}$<0.05) with the response not significantly different between the groups. Whereas, GFR in response to L-NAME decreased less in the uni-x as compared to the sham group (15 ± 4% vs 49 ± 3%, respectively, $P_{GroupXTreatment}$<0.0005), such that GFR was no longer different between the groups. Similarly, urine flow (UF) decreased significantly less in the uni-x as compared to the sham group (12 ± 0.15% vs 28 ± 0.5%, respectively, $P_{Group\ X\ Treatmenet}$<0.01).

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In conclusion, renal response to L-NAME infusion indicates that renal NO production maybe reduced in the female uni-x sheep, contributing to impaired renal function.

PII-172

Short Stature and Obesity in Women Living in Slums. João G. Alves, Renato A. Pinto, Romero W. Falcão. *Pediatrics, Instituto de Medicina Integral Prof Fernando Figueira (IMIP), Pernambuco, Brazil.*

To verify the association between height and overweight/obesity in women living in a slum.

A cross-sectional survey was carried out in a slum in Recife, northeast Brazil. It has an estimated population of 3733 people and the target population of this study consisted of 801 women aged between 20 and 60 years.

Weight as assessed with electronic scales and height was measured using a stadiometer. Women were considered to be of short stature if their height was equal to or lower than the 5th percentile of the height distribution of the Brazilian population of the same gender (149 cm).

Following training, community health workers administered the International Physical Activity Questionnaire (IPAQ) short form version and the 24-hour diet recall questionnaire in a weekday. For each food item, subjects identified serving size (small, medium, or large) and frequency of consumption. To help subjects estimate portion sizes, food pictures were used. Energy intake was calculated using Brazilian food composition tables.

Variables showing a p value < 0.20 in the bivariate analysis were included in the multiple regression analysis to assess association with overweight/obesity.

The study was approved by IMIP's Research Ethics Committee and participants gave written consent.

The prevalence of overweight/obesity was 45.5% ; respectively 28.7% and 16.8%. Physical inactivity was found in 17.4% women; 12.2% of the women had short stature and 43.7% had energy intake below the recommended dietary allowance. Bivariate analysis showed that overweight/obesity differed significantly in the following aspects: (i) short stature; (ii) abdominal waist; (iii) energy intake. The same variables remained statistically significant after adjusting in a multiple logistic regression model.

Short stature as a proxy of chronic undernutrition in early life was associated with overweight/obesity in this very poor women population. This finding is in agreement with Barker hypothesis and helps to explain the higher incidence of overweight/obesity in the poorest regions in developing countries.

PII-173

Smaller Pelvic Diameters in Pregnant Adolescents Contribute to Lower Birth Weight. Joao G. Alves, Luiza M. Mello, Lidia C. Siqueira, Jose N. Figueiroa. *Pediatrics, Instituto de Medicina Integral Prof Fernando Figueira (IMIP), Pernambuco, Brazil.*

To compare the pelvic diameters and birth weight among adolescents and adult pregnant women.

This cross sectional study enrolled adolescents and adult women who delivery at Instituto de Medicina Integral Prof. Fernando Figueira (IMIP)-Recife, Brazil - between January and December 2010. Written, informed consent was obtained from each subject before any procedures were carried out. The ethics committee at the IMIP approved the study.

It sampled 125 adolescents and 207 women with her newborns. Inclusion criteria were: adolescents (age between 10 and 19 years) and women with age between 20 and 45 years, primipara with a singleton pregnancy, a term gestation, and no maternal disease. It was excluded newborns with congenital infections, malformations or genetic syndromes.

Clinic pelvimetry was conducted by one trained researcher using the Collins pelvimeter. It was assessed the conjugate diameter, the intercristal diameter, and the interspinous diameter. Newborns were measured within the first 24h after delivery.

Associations between biological variables and continuous demographic and birth weight were assessed by likelihood ratio test, by adjusting the simple linear regression models. The effect of the independent variable teenage on the birth weight was evaluated using a set of multiple linear regression model.

The mean age was 16.8 years (SD = 1.6 ; 12 to 19 years) among the 125 pregnant adolescents and 27.3 years (SD = 5,2 ; 20 to 42 years) among the 207 adult pregnant women. There are no differences between gestational age. Adolescents mother show a lower pelvic diameters and shorter newborns.

Table. Characterisitcs of adolescents and adult mothers

	Adolescent	Adult	
	Yes (n =125)	No n =207)	Value p
Birth Weight	2990.0 (556.0)	3163.0 (513.0)	0.004
BMI (kg/m2)	24.2 (3.6)	26.4 (3.9)	0.001
School (years)	3.2 (0.6)	3.6 (0.9)	0.001
Diamter BC(cm)	22.4 (2.4)	24.1 (2.1)	0.001
Diameter BE(cm)	19.4 (2.4)	20.3 (2.5)	0.001
Diameter SPE(cm)	19.0 (2.3)	20.5 (2.5)	0.001
Gestacional age	38.9 (1.3)	39.0 (1.2)	0.467

Association tests between variables and birth weight showed that BMI, pelvic diameters and gestational age are associated with birth weight.

Ours results showed that a pelvis not completely developed diagnosticated by clinic pelvimetry contribute to restrain fetal growth in adolescents. This finding helps to explain the smaller birth weight among adolescents mothers.

PII-174

Accuracy of Compendium of Physical Activities-Predicted Energy Expenditure in Pregnant Women. Christina G. Campbell, Randal C. Foster, Lorraine M. Lanningham-Foster, Katie M. Smith. *Food Science and Human Nutrition, Iowa State University, USA.*

The Compendium of Physical Activities (CPA) provides the energy expenditure (EE) for hundreds of daily activities reported in metabolic equivalents (MET), defined either as the ratio of EE for an activity relative to the EE at rest or as the oxygen consumed with respect to a reference of 3.5 ml O2/kg/min. The CPA has been used to quantify the EE of activities in pregnant women even though changes associated with pregnancy may render the CPA values invalid. This study compares the energy cost of activities of daily living using indirect calorimetry (IC) in pregnant women to the METs in the CPA.

IC was used to measure EE in 17 pregnant women (10-14 weeks gestation) during simulations of activities of daily living (ADL) including rest, typing, standing while folding laundry, sweeping, and walking on a treadmill at 2.0, 2.5, 3.0 mph (0% incline) and 3 mph (3% incline). The MET value for each activity from the CPA was compared against two values derived from IC: the VO2 definition (standard MET) of the MET (3.5 ml O2/kg/min) and the quotient definition (measured MET) of the MET (kcal(activity)/kcal(REE)). Means for both comparisons were tested by one-sample t-test with a Bonferroni-corrected P value ($P < 0.007$). Residual analyses were constructed to examine other parameters, such as BMI.

Calorimeter MET values correlated with CPA MET values: standard MET vs. CPA $R^2 = 0.795$, $P < 0.001$, measured MET vs. CPA $R^2 = 0.712$, $P < 0.001$. The standard and measured MET values were highly correlated: $R^2 = 0.926$, $P < 0.0001$. There were significant differences between CPA and standard MET for rest and ADL EE ($P < 0.0005$), with CPA overestimating EE. The values for CPA vs. measured MET showed the same pattern ($P < 0.001$). Differences between standard MET and CPA during walking were not significant except at 3 mph ($P = 0.0013$), but differences between measured MET and CPA were significant ($P < 0.002$), with the CPA underestimating EE. Residual analysis showed that there were patterns in the errors between measurements: there was a correlation between the signed residuals and BMI across all activities, with increasing pre-pregnant BMI associated with CPA overestimation of energy expenditure.

The CPA can serve as an approximation of EE in pregnant women but its use may be limited by inaccuracy. Further study is required to obtain a more accurate assessment of EE in women throughout the course of pregnancy.

PII-175

Accuracy of Physical Activity Assessment during Pregnancy. Katie M. Smith, Randal C. Foster, Christina G. Campbell. *Food Science and Human Nutrition, Iowa State University, IA, USA.*

Prenatal physical activity can improve maternal and infant health and lower future disease risk for both mother and baby, however very few assessment methods have been validated for use during pregnancy. The purpose of this

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study was to evaluate the accuracy of a subjective physical activity record (PAR) and an objective activity monitor, against a reference standard based on participant interviews to quantify moderate and vigorous physical activity (MVPA) in pregnant women.

Fifty-two healthy pregnant women completed a physical activity record (PAR) and wore a SenseWear® Mini Armband (Model Name: MF) (SWA) activity monitor over a 7-day period at 18 weeks gestation. Total minutes spent in moderate and vigorous activity were totaled from both modalities and evaluated against the reference standard using contingency analysis and Pearson's chi-square test to evaluate the number of women meeting minimum physical activity recommendations (at least 3, 30 minute sessions of exercise per week). Both modalities were also tested individually and collectively to assess their ability as indicators of activity using empirically determined cut-offs as indicated by receiver-operator characteristic curves. These experimentally-derived criteria were also tested with Pearson's chi-square test.

Both the PAR and SWA overestimated exercise status when compared to the reference standard. According to the reference standard, 13 of 52 subjects (25%) met the criteria of 3, 30 minute sessions of volitional, moderate-intensity activity. Results from the PAR and the SWA determined that 42 and 52 subjects, respectively, achieved 90 minutes of moderate-vigorous physical activity (MVPA) ($P < 0.0001$ for both comparisons). Single-modality predictors of MVPA did not show a significant correlation. A composite predictor of MVPA offered the most favorable option for sensitivity and specificity (true positives, n=8 and true negatives, n=36) using cut-offs of 280 and 385 minutes/week for the PAR and SWA, respectively.

Compared to the reference standard, the cumulative time spent in MVPA obtained from the PAR or SWA overestimates the prevalence of women meeting prenatal exercise recommendations. The most accurate predictor of women meeting current prenatal exercise guidelines was identified by using the PAR and SWA collectively.

PII-176

Relationship between Sleep Quality and Mood Disturbances in Expecting Mothers during the Second Trimester. Robin Choo[1], Diaz Utama[2], Cornelia Chee[2], Helen Chen[3], Anne Rifkin-Graboi[1], Rui Kwan[2], Daniel Goh[2], Michael J. Meaney[1], Peter Gluckman[1], Seang Mei Saw[2], Yap Seng Chong[2], Kenneth Kwek[3], Joshua J. Gooley[4]. *[1]Singapore Institute of Clinical Sciences, Singapore; [2]National University Health System, Singapore; [3]KK Women's and Children's Hospital, Singapore; [4]Duke-NUS Graduate Medical School Singapore, Singapore.*

Poor sleep quality is associated with increased risk for mood disturbances including depression and anxiety. However, few studies have examined the relationship between sleep and mood during pregnancy, a time during which marked changes in sleep quality and mental health can occur. In the present study, we examined self-reported sleep and mood in 454 expecting mothers near the end of the second trimester.

Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI). Depression was assessed by the Beck Depression Inventory II (BDI-II) and the Edinburgh Postnatal Depression Scale (EPDS), and anxiety was examined using the State-Trait Anxiety Inventory (STAI).

Based on global PSQI scores, 45.5% of pregnant women experienced poor quality sleep during the second trimester (PSQI >5), and 31.8% reported sleeping less than or equal to six hours per night. Sleep quality correlated with depression scores on the BDI-II (Spearman's rho = 0.42, $P < 0.001$) and the EPDS (rho = 0.46, $P < 0.001$), such that expecting mothers who experienced poor sleep were more likely to report mood disturbances. The highest rated item on the BDI-II was a change in sleeping patterns. Even after removing this item from the analysis, a significant correlation was found for sleep quality versus adjusted depression scores (rho = 0.39, $P < 0.001$). Antenatal sleep quality also correlated with anxiety state (rho = 0.37, $P < 0.001$) and trait-like anxiety levels (rho = 0.34, $P < 0.001$).

These findings suggest that expecting mothers who experience sleep disturbances are more likely to feel anxious or depressed compared to pregnant women with good quality sleep. Given that sleep problems and mood disturbances are closely correlated, our results could have important implications for mental health care during pregnancy.

PII-177

Menarche as a Riskffactor for Hypertension in Pregnancy in the Western Region of São Paulo: The Butantã Cohort. Ana Maria U. Escobar[1], Maria Helena Valente[1], Maria Filumena S. Gomes[1], Leide irislayne M. Araujo[1], Rafael R. de Moraes[1], Luis Marcelo I. Cirino[2], Maria Tereza B. Fernandes[1], Isac de Castro[1], Alexandra Brentani[1], Sandra Josefina F.E. Grisi[1]. *[1]Pediatrics, Faculdade de Medicina da Universidade de São Paulo, SP, Brazil; [2]Surgery, Faculdade de Medicina da Universidade de São Paulo, SP, Brazil.*

Introduction: The onset of menarche is an important milestone in women's reproductive life and its earlier occurrence seems to be significantly related to a number of risk factors for chronic diseases such as hypertension and adult metabolic syndrome.**Objective**: To correlate the presence of hypertension during pregnancy with earlier menarche.

A longitudinal and retrospective study of 732 mothers and their infants followed at Butantã Cohort was conducted from January 2007 to December 2009. Butantã cohort is located in the western region of São Paulo City and is part of a research project of the Pediatrics Department. Patient's data were collected through a questionnaire that included information such as menarche onset, reproductive patterns, health history and morbidity during pregnancy, including hypertension. Association between menarche reported by mothers and positive history of hypertension during pregnancy was statistically analyzed. The cumulative frequency of menarche onset was sequentially analyzed with Pearson's chi-square test for independent groups and expressed proportions. Odds ratio for risk estimate was calculated.

When evaluating the correlation between hypertension and menarche onset age we observed a strong association; Odds = 2.5 (1.3 - 4.5), 31.48% (n=17) of women with menarche <= 10 presented high blood pressure during pregnancy vs 15.63% (n=106) with menarche onset > 11 years ($p = 0.0027$). In higher menarche onset cutoff scores, no association was found: from a sample of women who had menarche onset at 11 (20.25%) had hypertension during pregnancy, vs 15.85% women who had menarche at 12 ($p = 0.1903$), and its null Odds = 1, 34 (0.86 - 2.1). This pattern persisted for older age groups (menarche at 13 to 16).

The presence of reported hypertension during pregnancy was related to menarche onset before 11 years of age.

PII-178

Prevalence of Ante-Natal and Post-Natal Depression among Women Living in Slums in Mumbai, India. Meera Gandhi[1], Sujay Joshi[1], Ramesh Potdar[1], Harshad Sane[1], Caroline Fall[2]. *[1]Centre for the Study of Social Change, Mumbai, India; [2]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom.*

Post-partum depression (PPD) is a well-recognized complication of pregnancy. It has adverse effects on the well-being and development of the newborn. No previous studies have assessed the prevalence of PPD in deprived women living in urban Indian slums, or assessed whether it is purely a post-partum phenomenon or starts before the delivery. We aimed to measure the prevalence of ante-partum (APD) and post-partum depression among women enrolled in a large ongoing randomized controlled trial of a nutritional intervention to reduce low birth weight in slums of Mumbai [Mumbai Maternal Nutrition Project].

Depression was assessed using the Edinburgh Postnatal Depression Score (EPDS), a researcher-administered questionnaire method, in which a score of >10 represents possible depression, and a score of >13 represents probable depression. The questionnaire was translated into the local language (Marathi) and checked by independent back-translation. Participants were pregnant women registered with MMNP. To date, we have studied 419 women in their third trimester (29-32 weeks) and 174 women 30-45 days after delivery. Women were also asked questions related to obstetric history, socio-economic characteristics and problems like domestic violence, gender issues and stressful events.

55% (n=232) of ante-partum women and 53% (n=93) post-partum women had EPDS scores of >10. 29% (n=121) of ante-partum women and 26% (n=45) post-partum women had scores of >13. Percentages of the 132 women who had data at both stages were possible APD alone: 55.30% (n=73), possible PPD alone 55.30% (n=73) and both APD+PPD 37.87% (n=50). Equivalent figures for probable depression were 29.54% (n=39), 27.27% (n=36) and 17.42% (n=23). Poor family financial status was associated with

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a higher prevalence of possible PPD (59%). Lack of family support was not associated with an increased prevalence, though the study is ongoing and may lack power at this stage.
EPDS scores do not equate to a full clinical psychiatric diagnosis, but our data suggest a high prevalence of both APD and PPD among women living in Mumbai slums. Care givers need to be aware that depression can be an ante-natal as well as a post partum condition. Poverty appears to be a risk factor, and we will present further data on other socio-economic risk factors in the main presentation.

PII-179

Born with Low Birth Weight in Rural Southern India – What Are the Metabolic Consequences 20 Years Later? Nihal Thomas[1], Louise Groth Grunnet[2,3,4], Pernille Poulsen[5], Solomon Christopher[1], Rachaproleu Spurgeon[1], Mercy Inbakumari[1], Roshan Livingstone[1], Alexander George[1], Venkataraghava R. Mohan[1], Belavendra Antonisamy[1], Finney S. Geethanjali[1], Rajni Karol[1], Allan Vaag[3,4], Ib C. Bygbjerg[2]. *[1]1Department of Endocrinology, Diabetes and Metabolism, Christian Medical College, Vellore, India; [2]Department of International Health, University of Copenhagen, Denmark; [3]Steno Diabetes Center, Gentofte, Denmark; [4]Diabetes and metabolism, Rigshospitalet, Copenhagen, Denmark; [5]Medical & Science, GLP-1 & Obesity, Novo Nordisk A/S, Denmark.*
Low birth weight (LBW) is a risk factor for type 2 diabetes. Today Intensive metabolic examinations in LBW Asian Indians have almost exclusively been performed in heterogeneous urban immigrants outside India. Therefore the aim was to study the impact of low birth weight on adult anthropometry and glucose metabolism, including insulin secretion and action, in young, rural Indian men.
One hundred and seventeen young healthy men were recruited from a rural part in South Indian. Sixty one subjects were born with LBW and 56 were born with normal birth weight (NBW). Subjects underwent a hyperinsulinemic euglycemic clamp, intravenous and oral glucose tolerance tests and a DEXA scan. In addition, height, weight and glucose tolerance status was obtained from their parents.
Men with LBW were shorter (167±6.4 cm vs. 172±6.0 cm, $p<0.001$), lighter (51.9±9 kg vs. 55.4±7, $p=0.02$) and had a reduced lean body mass (42.1±5.4 kg vs. 45.0±4.5 kg, $p=0.002$) when compared to men with NBW. In addition, the men with LBW had a lower leg-to-total fat mass ratio, a tendency towards reduced bone mineral content and increased diastolic blood pressure. More subjects had impaired glucose tolerance in the LBW than in the NBW group. However, *in vivo* insulin secretion and peripheral insulin action was similar in the two groups. Parents, and in particular mothers, of the subjects with LBW were shorter than parents of subjects with NBW.
The confirmation of early though subtle features of the metabolic syndrome among young rural Indians with LBW support the idea of foetal programming contributing to the risk of type 2 diabetes and the metabolic syndrome. Although the absolute changes appear mild, factors such as urbanization, Westernization and ageing, may further aggravate and unmask the metabolic abnormalities linking type 2 diabetes and the metabolic syndrome with LBW in rural India.

PII-180

HbA1c for Diagnosis of Hyperglycemia in Epidemiological Studies. P. Hardikar[1], S. Joshi[1], D. Bhat[1], D. Raut[1], H. Lubree[1], P. Katre[2], A. Pandit[1], C. Fall[3], C. Yajnik[1]. *[1]Diabetes and Paediatrics Department, KEM Hospital & Research Centre, Pune, India; [2]Persistent Systems Ltd, Pune, India; [3]MRC Lifecourse Epidemiology Unit, Southampton, United Kingdom.*
HbA1c has been recommended for screening and diagnosis of diabetes, by the American Diabetes Association (ADA) and the World Health Organization (WHO). ADA proposed that HbA1c 5.7 - 6.4% is to be considered prediabetes and >6.5% diabetes. HbA1c could be a simpler alternative to an OGTT in epidemiological studies. However, HbA1c may be influenced by non glycemic factors including erythrocyte kinetics; for example: hemolytic anemias and iron deficiency. These may complicate the interpretation.
We examined the influence of glycemia and hematological parameters on HbA1c concentrations among young adults in a birth cohort, majority of whom had a normal glucose tolerance.
We measured HbA1c in 243 young adults (21y old) in the Pune Children Study using Bio-Rad D10 system, calibrated to NGSP. A 75g OGTT (WHO 1999) was used to assess glycemia. Total glycemia was calculated as fasting+30m+120m glucose. Hematological parameters were measured using Beckman Coulter AcDiff™(Miami, USA). Ferritin, vitamin B_{12} & folate were measured.
OGTT showed that 92% were normoglycemic and 5.7% prediabetic (0.4% IFG, 5.3% IGT) and 2.1% DM. Mean (range) for HbA1c was 5.4 (4.3–6.7)%, and by ADA criteria 21% were 'prediabetic' and 1% diabetic, giving a 47% sensitivity and 80% specificity compared to OGTT. Thirty four percent were anemic, 40% iron deficient (ferritin <15ng/mL), 38% B_{12} deficient (<150pM/L) and 17% folate deficient (<7nM). Forty nine percent of anemic had microcytic erythrocytes (MCV<80fL) and 1.6% had macrocytic erythrocytes (MCV>100fL).
On MLRA, higher HbA1c was predicted by higher glycemia (R^2 11.0%), higher red cell distribution width (R^2 9.8%) and lower mean corpuscular hemoglobin (R^2 2.7%), allowing for the effect of age, gender and BMI.
In another model, higher HbA1c was predicted by higher glycemia (R^2 11.0%), lower ferritin (R^2 7.8%) and higher BMI (R^2 1.4%), when hematological parameters were replaced by ferritin, B_{12} and folate, in the above model.
The unexpectedly high prevalence of 'prediabetes' by HbA1c measurements in young Indian subjects is partly contributed by hematological parameters (including iron deficiency). Given the high prevalence of iron deficiency in India and other low and middle income countries, utility of HbA1c to diagnose hyperglycemia needs to be further investigated.

PII-181

Quality and Quantity of Maternal Dietary Fat in Rats Alters Aorta Composition in the Adult Offspring. N. A. Irvine[1], C. Kelsall[1], C. Torrens[1], K. A. Lillycrop[2], M. A. Hanson[1], P. C. Calder[1], G. C. Burdge[1]. *[1]Faculty of Medicine, Southampton University, United Kingdom; [2]Faculty of Natural and Environmental Sciences, Southampton University, United Kingdom.*
In animal models maternal fat intake can alter vascular function in offspring. A maternal diet rich in saturated fat leads to abnormal aortic fatty acid composition and impaired small artery function in offspring[1]. Other dietary fats induce changes in endothelial function, modifying the risk of CVD. Endothelial cell function can be altered by changing the fatty acid composition, particularly the amounts of arachidonic acid (AA) and eicosapentaenoic acid (EPA) as these cause changes in eicosanoid release. However, it is unknown whether varying amount and type of maternal fat changes the fatty acid composition of the vascular system in the offspring and whether these are long-term changes.
Virgin female Wistar rats were fed 7% (w/w) or 21% (w/w) diets containing n-6 PUFA (safflower oil), *trans* fatty acids (hydrogenated soybean oil (HSO)), saturated fatty acids (butter) or EPA and Docosahexaenoic acid, DHA (fish oil) from two weeks prior to mating and throughout gestation and lactation. Postnatal day 28, offspring were weaned onto AIN93M (4% w/w soybean oil) and killed on postnatal d 77. Aortas were dissected, cleaned, frozen in liquid nitrogen and stored at -80°C. Aorta fatty acid composition was measured by gas chromatography[2]. Statistical analysis was performed using a general linear model with Tukey's *post hoc* testing.
There were interactive effects of sex and total maternal dietary fat intake (AA $p< 0.0001$; DHA $p= 0.001$), sex and fat type (AA $P = 0.002$; DHA $P = 0.007$) and total maternal dietary fat and fat type (AA $P = 0.036$; DHA $P = 0.04$). The main observed changes were in proportions of AA and DHA. Male offspring of dams fed a 7% diet of butter or HSO exhibited a decrease in AA, female offspring showed an increase in DHA. AA also increased in female offspring of dams fed butter. A maternal 21% diet caused a decrease in AA and DHA and increased α-linolenic acid in both male and female offspring irrespective of fat type.
Data show that both amount and type of maternal dietary fat induce long-term changes in the fatty acid composition of the aorta in adult offspring, contingent on sex. This may be a mechanism by which diet during pregnancy alters offspring endothelial function leading to vascular function changes.
[1]Ghosh *et al.*. (2001) *J Physiol* 533, 815-822
[2]Burdge *et al.* (2000) *Br J Nutr* 84, 781-787

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PII-182

Maternal Gestational Diabetes Mellitus and Risk of Childhood Overweight and Obesity in Offspring: A Systematic Review. Shin Y. Kim, Lucinda J. England, Andrea J. Sharma, Terry Njoroge. *Centers for Disease Control and Prevention, Atlanta, USA.*

We systematically reviewed research examining the association between gestational diabetes and childhood overweight and obesity. We summarized findings and addressed methodological limitations of previously published studies.

We identified studies from three sources: 1) a PubMed search of articles published between January 1990- January 2011, 2) reference lists of publications selected from the PubMed search, and 3) reference lists of review articles on gestational diabetes and childhood weight published in the last five years. We included cohort or case-control designed studies that reported gestational diabetes separate from pregestational diabetes, had a childhood overweight or obesity definition of BMI>85th or 95th percentile, and had a non-diabetic control group.

A total of 12 studies were included in the systematic review. The crude odds ratio for the relationship between gestational diabetes and childhood overweight or obesity ranged from 0.7 to 6.3 but in eight of the 12 studies associations were not statistically significant. In addition, the magnitude of the associations did not increase with increasing childhood BMI. Only three studies adjusted for any confounders. In the two that adjusted for prepregnancy obesity, the gestational diabetes and childhood overweight or obesity association was attenuated and not statistically significant. The third study compared treated and non-treated women with gestational diabetes and found the association with childhood overweight or obesity was not statistically significant among those who received treatment.

This review demonstrates that results of studies of the association between maternal gestational diabetes and offspring overweight and obesity have been suggestive but not consistent. Therefore, future research should address the severity and timing of exposure to maternal diabetes on overweight and obesity risk in offspring. In addition, research should assess the importance of confounders such as maternal prepregnancy body mass index, family history of diabetes, and maternal and infant lifestyle.

PII-183

Low Birth Weight and Insulin Resistance: Influence of Pre or Postnatal Stressors? Anita L. Kozyrskyj[1], Megan E. Alton[2], Brian J. MacNeil[3], Elizabeth A.C. Sellers[4], Allan B. Becker[5]. *[1]University of Alberta, Canada; [2]McGill University, Canada; [3]University of Manitoba, Canada; [4]University of Manitoba, Canada; [5]University of Manitoba, Canada.*

Small for gestational age (SGA) and preterm birth–appropriate for gestational age (PT-AGA) have increased the risk for insulin resistance and overweight in children, implicating *in utero* and *ex* utero programming. The HPA axis may be a pathway; cortisol and DHEA (dihydroepiandosterone) levels are higher in children born low birth weight children or exposed to pre/postnatal stress. Recently, insulin resistance in young adults has been linked to maternal prenatal stress but prenatal smoking has not been well-studied. We undertook an analysis of pre-adolescent children to determine the association between low birth weight and insulin resistance, and potential modification by pre and postnatal stressors.

Using data from the 1995 Study of Asthma Genes and the Environment birth cohort in Manitoba, Canada, SGA and PT-AGA were related to insulin resistance at age 10, using the homeostasis model for insulin resistance (HOMA-IR), based on fasting glucose and insulin levels. Cortisol and DHEA plasma levels were also measured. Linear regression models to predict log HOMA-IR were adjusted for age, gender, puberty stage, overweight, asthma status and breast-feeding duration. The modifying effects of cortisol/DHEA levels were also tested, as were pre and postnatal stressors: pregnancy smoking and maternal postnatal distress.

In this community sample of 342 children, 15% of children were born SGA (36-43 weeks) and 5% were PT-AGA (30-36 weeks). Mean HOMA-IR index values were 2.32 in PT-AGA children and 1.70 in all other children. Controlling for age, gender, puberty stage, cortisol/DHEA and asthma status, PT-AGA birth increased HOMA-IR indices by a factor of 0.75 ($p<0.006$) relative to normal birth weight. This association was found in overweight but not normal weight children. HOMA-IR was inversely related to cortisol levels and positively related to DHEA levels, but not to SGA. Maternal prenatal smoking slightly diminished the association of PT-AGA with HOMA-IR. It was enhanced following adjustment for maternal postnatal distress.

We found that PT-AGA birth elevated the risk for insulin resistance in overweight children at age 10, independent of puberty stage, stress markers and maternal postnatal distress. Modification of this association by pregnancy smoking also suggests an *in utero* programming pathway.

PII-184

Pilot Study: Maternal Betamethasone Administration Has Long Term Effects on Umbilical Cord Glucose Levels in Human Fetuses. M. A. Kutsche[1], A. Husar[1], L. Ehrlich[2], D. M. Sloboda[3,4], J. R.G. Challis[5], J. W. Dudenhausen[1], T. Harder[2], E. Beinder[1], T. Braun[1,2]. *[1]Department of Obstetrics, Charité Universitätsmedizin Berlin, Germany; [2]Division of Perinatal Programming, Charité Campus Virchow, Germany; [3]The Liggins Institute, University of Auckland, New Zealand; [4]The National Research Centre for Growth and Development, New Zealand; [5]Michael Smith Foundation for Health Research, Canada.*

We have previously shown that a single course of maternal betamethasone (BET) administration in pregnancy has been associated with decreased fetal birth weight and increased placental efficiency. Considering the fetal dependency on maternal glucose supply, the aim of this study is to investigate the role of glucose and impaired placental glucose transport as a cause for low birth weight after BET administration.

BET (single course, 2x12mg) exposed women (n=46) who delivered between 26 and 42 weeks of gestation were compared to gestational age-matched controls (n=50). Maternal and umbilical cord blood samples were obtained at the time of delivery. Maternal plasma glucose (MG) and insulin (MI) levels and plasma venous (VUG) and arterial (AUG) umbilical cord glucose levels were measured. Significance was accepted for $p<0.05$.

Maternal plasma glucose and insulin levels did not change significantly after BET treatment compared to controls, although a trend for increased glucose levels was noted in pregnancies with female fetuses ($p=0.06$). In male and female fetuses together, VUG and AUG were significantly increased after BET compared to controls. The ratios of MG and VUG; VUG and AUG; and MG and AUG in pregnancies with female fetuses, were significantly increased after BET exposure compared to controls.

Our data indicates that the effect of antenatal BET on plasma glucose levels was sex dependent. Particularly in pregnancies with female fetuses, the increased ratio of MG and VUG after BET suggests altered placental glucose transport at the time of delivery compared to controls. Additionally, an increase in the ratio between VUG and AUG indicates an increased fetal metabolism of glucose compared to controls. Early onset of fetal gluconeogenesis may also contribute to changes in fetal glucose plasma levels. Further studies are required to investigate the mechanisms of altered transplacental glucose transport after antenatal maternal BET administration and downstream effects on fetal growth and development.

PII-185

Oxidative Stress Programming in a Rat Model of Postnatal Early Overnutrition – Role of Insulin Resistance. Patricia C. Lisboa[1], Elaine Oliveira[1], Magna C.F. Passos[1], Ellen P.S. Conceição[1], Juliana G. Franco[1], Angela C. Resende[2], Taline A.S. Amaral[2], Egberto G. Moura[1]. *[1]Physiological Sciences Department, State University of Rio de Janeiro, Rio de janeiro, Brazil; [2]Pharmacology and Psicobiology Department, State University of Rio de Janeiro, Rio de janeiro, Brazil.*

Postnatal early overfeeding (EO) is related to later development of overweight and other metabolic disorders. As oxidative stress is implicated in most human diseases, as obesity and diabetes, we decided to study some parameters related to oxidative stress and insulin signaling in liver from EO animals in adult life.

To induce EO, litter size was reduced to three pups/litter (SL: small litter) and groups with normal litter size (NL:10 pups/litter) were used as control. After weaning, rats had free access to standard diet and water. Body weight and food intake were monitored daily and offspring were killed at 180 days-old (1 animal from each litter, n=8). Significant differences had $p<0.05$ or less.

As expected, SL rats had hyperphagia, higher body weight and higher visceral fat mass at weaning and adulthood. In liver, postnatal EO programmed for lower catalase (-42%), superoxide dismutase (-45%) and

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glutathione peroxidase (-65%) activities. The evaluation of liver injury in adult SL group showed lower nitrite content (-10%), higher liver and plasma malondialdehyde content (+25% and 1.1 fold-increase, respectively). No changes of total protein bound carbonyl or Cu/Zn SOD protein expression in liver were detected between the groups. Regarding insulin signaling pathway in liver, SL offspring showed lower IRβ (-66%), IRS1 (-50%), PI3-K (-30%) and Akt (-58%).
We evidenced that postnatal EO can program a higher oxidative stress in liver, caused at least in part by a lower enzymatic antioxidative defense, and insulin resistance may contribute for this impairment.

PII-186

Maternal and Fetal Sex Hormone Binding Globulin Concentration in Gestational Diabetes Mellitus and Normal Pregnancies. Bin Liu[1], Yun Xu[2], Jian-ming Liang[3], Huan-yu Xiao[4], Wei-yang Sheng[4], Yan-hong Sun[3], Zi-Lian Wang[1]. *[1]Department of Obstetrics and Gynecology, The First Affiliated Hospital of Sun Yat-sen University, Guangdong, China; [2]Department of Endocrinology, The Sixth Affiliated Hospital of Sun Yat-sen University, Guangdong, China; [3]Clinical Laboratory, The First Affiliated Hospital of Sun Yat-sen University, Guangdong, China; [4]Zhong-shan Medical School, Sun Yat-sen University, Guangdong, China.*
Sex hormone binding globulin (SHBG) is negatively correlated with insulin resistance (IR), and thereby serves as a biomarker for IR. In the present study, we investigated maternal and fetal SHBG levels in gestational diabetes mellitus (GDM) and normal glucose tolerance (NGT) pregnancy.
SHBG levels were compared between groups (GDM: n=29 vs. NGT: n=20). Then, the correlation between SHBG and clinical data, including maternal age, gestational age, height, pre-pregnant weight, pre-partum weight, as well as fetal gender, birth weight, birth height, and head circumference were analyzed. Additionally, in GDM group, the correlation between SHBG levels and laboratory data, including fast insulin,C-peptide,cholesterol, triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and H1Ac were analyzed.
Both maternal SHBG level in third trimester of pregnancy and fetal SHBG level were similar between groups. However, fetal SHBG level was negatively correlated with pre-partum BMI (R=-0.530, p<0.001), pre-partum weight (R=-0.463, p=0.001), pre-pregnant weight (R=-0.326, p=0.022), and weight gain in pregnancy (R=-0.390, p=0.006) in the whole analyzed population. In addition, fetal SHBG level was negatively associated with lipo-protein a (R=-0.470, p=0.010) and H1AC (R=-0.437, p=0.018) in GDM groups.
These results indicated that, maternal overweight may lead to fetal IR, and good control of lipid and long term blood glucose levels in GDM mother would relieve fetal IR to some degree.

PII-187

VLDL-Specific Hypolipidemia Pattern in Human Subjects with Autism and Autistic Rodent Models. Hideo Matsuzaki[1], Keiko Iwata[1], Kenji J. Tsuchiya[1], Hiroaki Itoh[2], Norio Mori[1]. *[1]Research Center for Child Mental Development, Hamamatsu University School of Medicine, Japan; [2]Perinatal center, Hamamatsu University School of Medicine, Japan.*
The neurobiological basis for autism remains poorly understood, but evidence is mounting in support of lipid metabolism playing a role in autism. In order to clarify the role of lipids in autism, we examined serum lipid profiles of human subjects with autism and autistic rodent models.
This study enrolled 112 subjects with high-functioning autism recruited from the Asperger Society Japan and 106 age-matched healthy control subjects recruited by advertisement. All participants for both groups are Japanese male. In animal model experiment, valproic acid (VPA) exposed model mouse (Kolozsi *et al.* 2009) and CD38 null mouse (Jin *et al.* 2007) were used as autistic rodent models. Fasting human blood samples were collected by venipuncture in a sitting position with a tourniquet from all participants between 8:00 and noon. Mice were anesthetized by diethyl ether and then its blood samples were collected from the left ventricle. All blood samples were kept at room temperature for 30 min and centrifuged at 2000g for 10 min in a refrigerated centrifuge. After that, they were divided into 200-µl of aliquots and stored at -80°C for subsequent analyses. The size distribution of serum lipoprotein particles was evaluated by high sensitivity lipoprotein profiling system with high-performance liquid chromatography (Skylight Biotech, Inc., Akita, Japan).
The serum levels of total cholesterol and triacylglycerol in the infant subjects (under 20 years old) with high-functioning autism were significantly lower (Mann-Whitney U test: $p < 0.001$) than those of normal control subjects. In each fraction, there were significant differences in the serum levels of very-low density lipoprotein (VLDL) and high density lipoprotein (HDL) fraction. In particular, it's remarkable in VLDL fraction of triacylglycerol ($p< 0.00003$). However, there were no differences between the patients with autism and healthy subjects in serum chylomicron and low density lipoprotein (LDL) levels. The VPA-exposed mice and CD38 null mice in four weeks old have also shown serum lipid profile as above.
The association between autistic phenotype and abnormal serum lipid profile in human subjects and rodent models suggests that individuals with autism may be at increased risk for VLDL hypolipidemia in infancy and which might be implicated in the pathophysiology of autism.

PII-188

Pregnancy Associated Changes in Insulin Signalling in Daughters of Adolescent Ewes. M. H. Oliver[1,2], S. N. Hancock[1,2], P. R. Kenyon[1,3], H. T. Blair[1,3], S. Pain[1,3], S. Morris[1,3], H. H. Phua[1,2], F. H. Bloomfield[1,2]. *[1]Nat Res Ctr Growth & Devel, Univ Auckland, New Zealand; [2]Liggins Inst, Univ Auckland, New Zealand; [3]Massey Univ, New Zealand.*
In humans, offspring of adolescent mothers are at increased risk of reduced birth weight and preterm birth. The effect of being born to an adolescent mother on the female offspring's metabolic adaptation to her own pregnancy is unknown. Aims/Hypothesis: Determine the effect grand-maternal (G0) age at pregnancy has on muscle insulin signalling proteins in G1 daughters before and during mid pregnancy.
Singleton offspring born to 3-4yr old mature (Mat, n=11) or eight month old immature (Imm, n=8) ewes were mated at 18 months of age to the same rams. Immediately following GTT (0.5g/kg glucose iv) before pregnancy and at 60d, term=147) muscle biopsies were taken for Western Blot analysis of insulin receptor β sub unit (IR-β), insulin receptor substrate protein 1 (IRS-1), regulatory subunit p85α (p85), protein kinase Cζ (PKCζ) and glucose transporter 4 (Glut4). Data are mean±SEM.
Imm G0 ewes were 36% lighter than Mat ewes at mating ($p<0.0001$) and had singleton offspring 20% lighter at birth ($p<0.01$). G1 weights were identical at mating (51±1kg). Glucose area under the curve tended to be lower in Imm than Mat ewes before G1 pregnancy (1619±46 vs. 1740±52 mM.min, $p=0.1$) but not at d60 of pregnancy (1640±48 vs 1673±56 mM.min). Insulin responses were not different between groups. In Imm ewes IRS-1 and Glut4 protein expression decreased by 30% with pregnancy (both $p<0.05$) but there were no similar changes in Mat ewes. In both groups, p85 protein expression decreased by 15% with pregnancy ($p<0.05$) while in Mat ewes PKCζ protein expression increased 20% ($p<0.05$). IR-β was not affected by pregnancy in either group. Birth weights of G2 lambs were not different between groups.
The data from these studies suggest that daughters of ewes mated in adolescence have made a functional adaptation in muscle insulin signalling activity by mid G1 pregnancy to relax glycaemic control to favour substrate availability for conceptus development. These effects were independent of effects on insulin secretion or IR-β expression. Despite the large difference in grand maternal weight (G0) at mating and reduced G1 size at birth, functional adaptation of insulin signalling protein expression has helped normalize birth weight of G2 lambs. It remains to be seen if these findings are translatable to humans.

PII-189

Hypoxia-Induced Alterations in Skeletal Muscle Cell Respiration. Kathleen R. Belgrave, James Staples, Timothy Regnault. *Biology, University of Western Ontario, ON, Canada.*
Intrauterine-growth restriction (IUGR) is a condition where fetuses fail to reach their genetic growth potential as a result of placental insufficiency. These fetuses commonly develop in a hypoxic in utero environment and IUGR offspring display impaired skeletal muscle mitochondria biogenesis. These alterations closely mimic symptoms observed in adult patients with insulin resistance. This supports the concept that in utero changes

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in mitochondrial function could become permanent, predisposing IUGR individuals to metabolic diseases such as Type 2 Diabetes. One pathway potentially altered in IUGR predisposing mitochondrial dysfunction is the peroxisome proliferator-activated receptor gamma co-activator-1α (PGC-1α) pathway. PGC-1α is a transcriptional co-regulator for the expression of genes for mitochondrial biogenesis and respiration. Using the C2C12 mouse muscle myoblast cell line, we propose that cells which differentiate from myoblasts to myotubes under hypoxic conditions will display decreased mitochondrial respiration and mRNA levels of markers of respiration and biogenesis (PGC-1α, PPAR-α, -β, RXR-α, -β); predicted to persist following a return of cells to normoxic conditions.

C2C12 cells were incubated at 1% (IUGR conditions), 5% (fetal normoxia) or 20.9% (normoxia) oxygen concentration for five days. After five days of differentiation, a subset of each oxygen treatment was placed at control (20.9% oxygen, recovery) for an additional two days. Cells were collected at Day 0 (before differentiation), Day 5 (differentiation for five days) and Day 7 (recovery) for cellular respiration and mRNA analysis.

A decrease in cellular respiration in the 1% oxygen treatment compared to the 5% and 20.9% oxygen treatments was observed after five days of differentiation and post-recovery period ($p < 0.05$). This indicates that hypoxia-induced alterations to the pathway may be persisting after the hypoxic insult, modulating oxygen consumption. This is further confirmed by the trend of PGC-1α mRNA levels which remain decreased after both five days under hypoxia and a two day recovery period.

Hypoxia, a major contributor to IUGR, may induce permanent changes in mitochondrial respiration and biogenesis. This suggests IUGR offspring may be predisposed to insulin resistance in skeletal muscle.

PII-190

Metabolic Imprinting Effect in Beef Production: Effects of Nutrition Manipulation during an Early Growth Stage on Fatty Acid Composition in Longissimus Muscles of Wagyu (Japanese Black). Takafumi Gotoh[1], Kotaro Etoh[1], Kunihiko Saitoh[2], Kaoru Saitoh[2], Hironori Sakuma[2], Kaori Sakuma[2], Syuichi Kaneda[2], Tsuyoshi Abe[2], Tetsuji Etoh[1], Yuji Shiotsuka[1], Kenichiro Matsuda[1], Hidetoshi Suzuki[2], Hiroyuki Hasebe[2], Fumio Ebara[1], Akira Saitoh[3], Jochen Wegner[4]. *[1]Kyushu University, Oita, Japan; [2]National Livestock Breeding Center, Japan; [3]Zen-Raku-Ren, Japan; [4]Leibniz Institute for Farm Animal Biology, Germany.*

The aim of this study was to investigate, using gas chromatography analysis, whether the metabolic imprinting effect of differences in feeding during an early growth influences on the fatty acid composition in LM of Japanese Black steers or not.

The high energy group (HE: n=12) underwent intense nursing (milk replacer: crude protein 26%, crude fat 25.5%, maximum intake of 1.8 kg per day) at three months of age and was fed a high-concentrate diet for four to 10 months of age. On the contrary, the roughage group (R: n=11) underwent normal nursing (the same milk replacer, maximum intake of 0.6 kg per day) and was fed only roughage *ad libitum* from four to 10 months of age. After 10 months of age, both groups were fed only roughage *ad libitum* and grazed from 11 to 31 months of age. Steak samples of the longissimus muscles (LM) in all animals were collected from carcasses after slaughter. Intramuscular fat contents and fatty acid compositions were analysed by gas chromatography method and Soxhlet methods, respectively. Moreover the concentration of vitamin E in LM was measured by HPLC.

Intramuscular fat content of LM was significantly larger in group HE (13.2%) than in group R (9.4%) ($P < 0.05$). Muscle from groups HE contained much higher proportions of the fatty acids C18:1, C18:2, C20:1, unsaturated fatty acid, monounsaturated fatty acid than those from group R ($P < 0.01$). Concentration of vitamin E in LM was larger in group HE than in group R ($P < 0.01$).

This may be caused by the effect of metabolic imprinting induced by a high feeding level during the early growth stage.

PII-191

The Role of Hypoxia and Telomerase on Senescence in Fetal Guinea Pig Muscle Cells. Stephanie E. Hallows, Timothy R.H. Regnault, Dean H. Betts. *Physiology and Pharmacology, University of Western Ontario, ON, Canada.*

Low birth weight infants have a higher risk of developing metabolic syndrome, including Type II Diabetes. Fetal muscle can be severely growth restricted in low birth weight infants and is the main tissue for determining insulin resistance. Recent studies indicate accelerated senescence can occur in organs of low birth weight rodents, which could lead to adult disease. Hypoxic in utero environments may play a role in the development of senescence through increased stress and reactive oxygen species (ROS). Telomerase, which is present during development, may play a role in protecting cells from oxidative stress. We postulate that hypoxic environments will induce an increase in senescent markers along with elevated oxidative stress and ROS levels, and that telomerase may have a protective role in the cell.

A primary cell line of fetal guinea pig myoblasts were grown at various oxygen partial pressures of 20%, 5%, 2% and 1%. Cells were fixed with 4% paraformaldehyde, blocked and incubated for one hour each with primary and secondary antibodies for immunocytochemistry. Cells were collected after a four day differentiation period and prepared for western blotting. ROS detection was also conducted with DCF and MitoSOX and analyzed by flow cytometry. Additionally cells were prepared as above and the effects of the Telomerase Inhibitor IX (Calbiochem) in normoxic and hypoxic conditions and collected for western blotting and a measurement of telomerase activity.

Cells were treated with telomerase inhibitor and its activity was significantly decreased by 60% at 20% PO2. Cells in normoxia and hypoxia showed decreased growth but to a lesser extent in 2% PO2 compared to 20% PO2. This was accompanied with a dose dependent increase in senescent markers of p21, p53 and an altered metabolism with similar levels of mTOR and S6K protein but show a 50% increase in phosphorylation of both at 2% PO2 compared to 20% PO2. The mTOR pathway and oxidative stress are both linked to p66shc, whose phosphorylation is increased at 2% PO2 also.

Cells grown at 2%O2 display higher growth rates and less senescent markers when treated with a telomerase inhibitor. The role of telomerase in these cells may have a protective role in preventing senescence through telomere end maintenance or preventing oxidative stress. Further studies will need to be done in true hypoxic environments and verifying the effects in in vivo models of intrauterine growth restriction guinea pigs.

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No Association between Body Size at Birth and Leukocyte Telomere Length in Adult Life – Evidence from Three Cohort Studies. Eero Kajantie[1,2], Kirsi H. Pietiläinen[3,4], Karoliina Wehkalampi[1,2], Laura Kananen[5,6], Katri Räikkönen[7], Aila Rissanen[4], Petteri Hovi[1,2], Jaakko Kaprio[1,3], Sture Andersson[2], Johan G. Eriksson[1,8], Iiris Hovatta[1,5,6]. *[1]National Institute for Health and Welfare, Helsinki, Finland; [2]Children's Hospital, University of Helsinki, Finland; [3]Twin Research Unit, Hjelt Institute, University of Helsinki, Finland; [4]Obesity Research Unit, Helsinki University Central Hospital, Finland; [5]Research Programs Unit, Molecular Neurology, Biomedicum Helsinki, University of Helsinki, Finland; [6]Department of Medical Genetics, Haartman Institute, University of Helsinki, Finland; [7]Department of Behavioural Sciences, University of Helsinki, Finland; [8]Department of General Practice and Primary Health Care, University of Helsinki, Finland.*

A short leukocyte telomere length (LTL) is a promising overall marker of ageing. Shorter LTL in adult life is predicted by adverse early life conditions such as child maltreatment, but associations with fetal life conditions are not known. We studied whether markers of fetal and neonatal conditions predict LTL in adult life.

We used three complementary cohorts: (a) 1894 subjects from the Helsinki Birth Cohort Study (HBCS), aged 56 to 69 y representing the whole normal variation in fetal growth; (b) The Helsinki Study of Very Low Birth Weight Adults (HeSVA) encompassing 334 subjects aged 18 to 27 y: 164 born preterm at a very low birth weight (VLBW; <1500 g; representing extreme pre- and neonatal conditions) and a comparison group of 170 term-born

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controls; (c) 248 twins aged 23 to 31 y (41 MZ and 83 same-sex DZ pairs) from the FinnTwin16 cohort. We measured telomere length from peripheral blood cells by real-time quantitative PCR.
Shorter LTL was associated with higher age in HBCS and among men of the HeSVA. It was also associated with lower childhood socio-economic status in the HBCS and FinnTwin16 and with lower maternal age in the HBCS. LTL was not associated with weight, length, ponderal index or gestational age at birth in any cohort. LTL was similar in subjects born at VLBW and controls born at term. LTL was correlated within twin pairs (intra-class correlations 0.76 for MZ and 0.39 for DZ) but within-pair differences in LTL were unrelated to those of the birth measurements.
LTL in adult life is not associated with body size at birth, length of gestation or preterm birth at VLBW. It is thus unlikely to be a mechanism linking fetal and neonatal conditions with individual differences in ageing.

PII-193

Maternal Vitamin D Deficiency Effects Offspring Bone Quality throughout the Lifecourse. Stuart Lanham[1], Carol Roberts[1], Angela Habgood[1], Suzanne Alexander[2], Thomas H.J. Burne[2], Darryl W. Eyles[2], Clive N. Trueman[3], Matthew Cooper[3], Cyrus Cooper[1], John J. McGrath[2], Richard O.C. Oreffo[1]. *[1]Bone and Joint Research Group, University of Southampton School of Medicine, United Kingdom; [2]Queensland Centre for Mental Health Research, University of Queensland, Australia; [3]School of Ocean and Earth Science, University of Southampton Waterfront Campus, United Kingdom.*
Animal models suggest vitamin D is not essential for fetal bone development, but is necessary postnatally for calcium metabolism.
We have used a rat model to determine if maternal vitamin D deficiency altered bone structure and development in offspring at various ages. Dams were maintained under UV deplete conditions and fed a vitamin D deficient diet from four weeks of age. They were mated at 10 weeks of age. At birth, all mothers were fed a vitamin D replete diet. Offspring were tested at birth, 21 days of age (weaning), 70 and 140 days of age. Offspring bones were analysed for structure, density, strength and composition using micro-computed tomography, 3-point bend testing, and plasma mass spectrometry.
At birth, developmentally vitamin D-deficient (DVDD) offspring had vertebra with lower BV/TV due to reduced trabecular thickness and increased trabecular spacing. At 21 days of age, there was reduced trabecular thickness in the proximal and distal tibia. At 70 days of age, additional structural differences were observed in the proximal tibia. At 140 days of age, the femoral head region showed structural differences including reduced trabecular thickness. Following analysis by gender, the male DVDD offspring showed altered vertebra bone structure at birth, but relatively few differences at the other timepoints. In contrast, the DVDD females showed few differences at birth, but increased differences at 21 and 70 days of age. The femoral head differences observed at 140 days occurred in the DVDD female offspring and not males. Furthermore, male and female DVDD offspring showed increased levels of mineralization in the secondary ossification centre of the femoral head. At 21 days of age, no differences were found in the levels of calcium, strontium, or barium in the femur between controls and DVDD offspring. However, at 140 days of age, the Sr/Ca ratio was higher in the femoral head and midshaft of the DVDD offspring.
These results illustrate that vitamin D depletion in utero does have long lasting consequences on the bones of related offspring.

PII-194

The Role of Thyroid, Adrenal and Pancreatic Hormones on Fetal Bone Development. Stuart Lanham[1], Abigail L. Fowden[2], Dominique Blache[3], Carol Roberts[1], Cyrus Cooper[1], Richard O.C. Oreffo[1], Alison J. Forhead[2]. *[1]Bone and Joint Research Group, University of Southampton School of Medicine, United Kingdom; [2]Department of Physiology, University of Cambridge, United Kingdom; [3]Faculty of Natural and Agricultural Sciences, University of Western Australia, Australia.*
Little is known regarding which hormones are important for bone development before birth. However, there is increasing evidence that bone is also an endocrine organ in adults[1,2,3].
Using a fetal sheep model, we used computed-tomography and mechanical testing to assess the relative importance of thyroid, adrenal, and pancreatic hormones in the fetal development of the metatarsal bone in the third trimester. In addition, we determined plasma levels of total calcium, leptin, and indicators of osteoblast (osteocalcin) and osteoclasts activity (C-terminal telopeptides of Type I collagen - CTX).
Fetal thyroidectomy (TX) produced significant dramatic alterations in bone development, as well as low levels of T_3, T_4, and osteocalcin, and increased levels of insulin and leptin. Fetal pancreatectomy (PX) produced thicker trabeculae, low levels of T_3, insulin, leptin, and CTX. In contrast, fetal adrenalectomy (AX) showed no apparent alteration to bone development, but did produce low levels of cortisol, T_3, and leptin. Hence, PX effects appear to be due to low insulin and low CTX, and TX effects due to low T_4 and osteocalcin, and high insulin and leptin. Low insulin levels are known to increase osteoprotegerin levels and inhibit osteoclast activity[1] explaining the PX results. High leptin levels, seen in TX samples, inhibit bone formation[2] and insulin secretion through a neuronal relay[3]. In addition, insulin signalling in osteoblasts acts upstream of osteocalcin in a positive feedback loop to increase osteocalcin levels[1]. However, TX samples displayed high insulin levels with high leptin levels and low osteocalcin levels, suggesting bone endocrinology is altered in the fetus.
These studies demonstrate the importance of endocrine hormones in fetal skeletal development and have implications for bone physiology.
References:
1. Ferron *et al.* Cell 2010; 142: 296-308.
2. Ducy *et al.* Cell 2000; 100: 197-207.
3. Hinoi *et al.* J Cell Biol 2008; 183: 1235-1242.

PII-195

Changes in Chick Cerebellum after Antithyroid Drug Administration during Prenatal Period. Gen Haba[1], Hidekazu Nishigori[2], Makoto Sasaki[1], Koujiro Tohyama[1], Toru Sugiyama[1], Keisuke Kagami[3], Hideo Nishigori[3]. *[1]Iwate Medical University School of Medicine, Iwate, Japan; [2]Tohoku University Hospital, Miyagi, Japan; [3]Iwate Medical University School of Pharmacy, Iwate, Japan.*
Chick brain is quite well developed at the time of hatching under thyroid hormone (TH)actions. This physiological situation is more comparable to humans than rodent wherein THs are thought to act on the brain mainly after birth. To elucidate one of the possible relationships between prenatal hypothyroid state and congenital hypothyroidism, we examined the cerebellum of hatched chick after the treatment of methimazole (MMI) during embryogenesis.
Fertilized eggs treated with MMI (20 μmol/egg) or vehicle on day 14 were incubated at 37.5°C and 68% relative humidity. Embryos treated with MMI required additional three days for hatching (on day 24) compared with control (on day 21).
1) Compared to the controls, the hatched MMI-treated chicks appeared externally indistinguishable but exhibited heavier in brain parts and poorer scores of imprinting abilities. 2) The brains of hatched chicks under anesthesia were examined by 3T-MRI. Quantitative measurement revealed that the areas of the optic lobe, cerebellum and brain stem in the MMI-affected brain were significantly large and ADC was utilized to determine the increased space. 3) By histopathological examinations of the cerebellum, the MMI-affected cerebellum exhibited increased weight and size, and was enhanced immunoreactivity against GFAP suggesting the increase of astrocytes. By using the calcium-binding protein Calbindin as a marker of Purkinje cells, in the cerebellum, Purkinje cell arborization as well as granule cell migration was almost complete. However, the hypothyroid state caused by MMI disturbed the development of Purkinje cell processes and produced morphological changes in dendritic arborization or hypoplasia of the cells. 4) By a proteomic analysis, the quite different patterns between the control and MMI-affected cerebellum were observed.
Our data suggested that TH was involved in proper development of the cerebellum of chick embryos and that the delayed morphogenesis and changed profiles of the cerebellar proteins during prenatal hypothyroid state probably influenced physiological and behavioral activities in later life. Experiments were carried out with the approval of Institutional Animal Care and Use Committee. .

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Gene Network of Fetal Brain Reacts to Maternal Undernutrition Like-Hypoxia. Takuya Ito[1], Yoshitaka Kimura[2], Kaori Tanabe[2], Ai Nakamura[2], Kiyoe Funamoto[2], Ayako Aoyagi[2], Kazuyo Sato[3], Tetsuro Hoshiai[3], Kaori Suenaga[3], Junichi Sugawara[3], Satoru Nagase[3], Kunihiro Okamura[4], Nobuo Yaegashi[3]. *[1]Innovation of New Biomedical Engineering Center, Tohoku University, Japan; [2]International Advanced research and Education Organization, Tohoku University, Japan; [3]Division of Obstetrics and Gynecology, Tohoku University, Japan; [4]KKR Tohoku Kousai Hospital, Japan.*

It is known that maternal undernutrition during pregnancy is a factor inhibiting fetal growth and inducing cardiovascular diseases and neuropsychiatric disorder. However, the mechanism is complicated and still unknown. We have designed an experimental model of undernutrition in pregnant mice to evaluate the background gene network shift in murine fetal brain by maternal undernutrition.

Female C57BL/6N mice were given low protein (L) or regular (N) food two weeks before mating and during their pregnancy. The fetal hearts were collected on day 17.5 of gestation, which was late pregnancy, to evaluate effercts of maternal undernutrition on overall gestation period.

Network of hypoxia-inducible factor 1 alpha (HIF1a and prolyl hydroxylases: PHD1, HIF1a inhibitory regulator, and mammalian target of rapamycin: mTOR, HIF1a stimulatory regulator) were assayed by real-time quantitative PCR (qPCR), and evaluated in protein levels of HIF1a by immunohistochemistry.

qPCR showed the mRNA expression levels of both HIF1a and mTOR of fetal brain were increased by maternal undernutrition. In immunohistochemistry, HIF1a positive cells were observed in subventricular zone with maternal under-nutrient condition.

Increase in the expression of HIF1a is seen in hypoxia. In this study, HIF1a incerased and HIF1a network was activated without hypoxia in fetal brain by maternal undernutrition. In contrast, increase in HIF1a in tumor has been reported even in normoxia. In this study, HIF1a was increased without hypoxia in fetal heart by maternal undernutrition suggesting that maternal undernutrition induced hypoxia-like gene network responses in fetal brain.

PII-197

DNA Methylation Profiling Identifies Profound Epigenetic Differences between Hypothalamic Neurons and Glia. Ge Li[1], Wenjuan Zhang[1], Eleonora Laritsky[1], Maria S. Baker[1], Robert A. Waterland[1,2]. *[1]Department of Pediatrics, Baylor College of Medicine, USDA/ARS Children's Nutrition Research Center, TX, USA; [2]Department of Molecular and Human Genetics, Baylor College of Medicine, TX, USA.*

Epigenetic regulation in the hypothalamus could be altered by environmental factors during development, persistently influencing hypothalamic function and obesity susceptibility. Epigenetic characterization of the hypothalamus is complicated by the presence of two cell types – neurons and glia – which potentially have very different epigenetic regulation. The aim of this study is to perform genome-scale DNA methylation profiling to compare hypothalamic neurons and glia.

Hypothalamic nuclei were isolated from 21-day old male inbred mice and separated via cytometry with the neuron specific nuclear marker NeuN. Separation of neuronal and glial nuclei was confirmed by measuring expression of neuron or glia specific genes. DNA methylation profiling was performed with methylation specific amplification microarray (MSAM) with two biological replicates, each representing a pool of six mice. Methylation differences were valided by bisulfite pyrosequencing.

Clean separation of neuronal and glial nuclei was indicated by the distinct peaks during sorting, and confirmed by the expression of *Tubb3* and *Gfap* in the appropriate fractions. MSAM identified 1473 genomic regions with lower methylation in glia than in neurons and 375 vice versa. Interestingly, regions with lower methylation in glia tended not to be associated with genes ($P<10^{-4}$). Regions with lower methylation in neurons were enriched at gene promoters ($P<10^{-4}$). Promoters with lower methylation (i.e. more likely to be expressed) in glia were significantly ($P<10^{-3}$) enriched in 26 gene ontology categories including "response to stimulus" ($P<10^{-5}$) and "extracellular region" ($P<10^{-7}$); those in neurons were enriched in three categories ($P<10^{-3}$) including "K-transporting ATPase activity" ($P<10^{-3}$). Of five genes (*Bmp2*, *Bmp4*, *Bmp7, Atp1a1*, and *Atp1a3*) selected from the categories above for validation by bisulfite pyrosequencing, 100% validated. Association of the DNA methylation with gene expression is being investigated.

DNA methylation profiles in hypothalamic neurons and glia are remarkably different. Understanding the role of early environment in epigenetic dysregulation and subsequent obesity will therefore likely require analyzing separately induced epigenetic alterations in hypothalamic neurons and glia.

PII-198

Effects of Corticosteroid Treatment Either in Early or Late Pregnancy Restricts Brain Growth in Fetal and Postnatal-Age Sheep. Shaofu Li[1], Deborah M. Sloboda[2], Timothy J.M. Moss[3], Ilias Nitsos[3], Graeme R. Polglase[3], John P. Newnham[1], John R.G. Challis[4]. *[1]School of Women's and Infants' Health, The University of Western Australia, WA, Australia; [2]Liggins Institute, and The National Research Centre for Growth and Development, The University of Auckland, New Zealand; [3]The Ritchie Centre, Monash Institute of Medical Research & Dept of Obstetrics & Gynaecology, Monash University, Australia; [4]Michael Smith Foundation for Health Research, Canada.*

Aim: The aim of this study was to determine the effects on brain growth in fetal and postnatal sheep of corticosteroids given to their mothers either in early or late gestation.

Methods: Cohort 1: pregnant ewes carrying singleton fetuses were randomized to control (2ml saline/ewe) or dexamethasone (dex) treatment (0.14mg/kg ewe weight) consisting of four intramuscular injections at 12-hourly intervals over 48 hours on days 40-42. Brains were collected from the offspring at seven months postnatal age. Cohort 2: pregnant ewes carrying singleton male fetuses were injected with 1 (104 days of gestation; dG), 2 (104, 111 dG) or 3 (104, 111, 118 dG) doses of betamethasone (beta; 0.5mg/kg body weight) or saline (control). The brain was collected and weighed (whole and in sections) at 75, 84, 101, 109, 116, 122, 132, 145 dG and in offspring at six and 12 weeks (wks) of age.

Results: Cohort 1: the cerebellum weights of dex males were lower than control males (p=0.037). Pituitary weights of dex females were lower than control females (p=0.045). There was no difference in the total brain or hippocampal weights between dex and control groups and no effect of sex. Cohort 2: total brain weights of beta group were lower than control groups from 109 dG to six wks postnatal age ($p\leq 0.03$). The cerebellum weights were lower from 109 to 145 dG ($p\leq 0.04$). The hippocampal weights at 132 dG (p=0.003) and pituitary weight at 122 and 132 dG ($p\leq 0.02$) were reduced by beta. The ratio of total brain, cerebellum, hippocampal and pituitary weight to body weight were not significantly different on two cohort experiments.

Conclusions: Administration of corticosteroids to early or late pregnant sheep retarded fetal and postnatal brain growth. Late gestation corticosteroid treatment appears to have greater effects on the brain and various components. Exogenous corticosteroids alter brain development in the developing fetus.

PII-199

Diencephalic Expression of Angiotensin and Glucocorticoids Signaling Proteins in Maternal Undernutrition Male Offspring. Marcelo Cardoso de Lima[1], José Eduardo Scabora[1], Flávia F. Mesquita[1], Agnes Lopes[2], Daniele B. Torres[2], Ize P. de Lima[1], José Antonio R. Gontijo[1], Patricia Aline Boer[2]. *[1]Department of Internal Medicine, School of Medicine, 6111, UNICAMP, Campinas/SP, Brazil; [2]Department of Morphology, IBB, UNESP, Botucatu/SP, Brazil.*

The current study investigates whether gestational undernutrition alters the cerebral expression and localization of AngII receptors (AT1R/AT2R), AVP, POMC, ACTH, and steroid receptors (MR and GR) in offspring.

Virgin female Wistar rats were fed during pregnancy with normal-protein (NP 17% casein, n=6) or protein-restricted diets (LP 6% casein, n=6). The male pups were followed and maintained with normal chow until adulthood. Cerebral tissues were obtained from male Wistar offspring of time-mated rats at 16 week-old. Protein expression of AT1R and AT2R and, AVP, POMC, ACTH, MR and GR was measured by immunoblotting and immunohistochemistry.

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The blood pressure increased significantly in LP rats, from 116.2±6.5 mmHg to 137.9±6.9 mmHg, p<0.01) as compared with a smaller and non-significant rise from 114±7.4 mmHg to 128.8±8.7 mmHg in NP at 16 weeks of age. Western blot analysis in male offspring of NP and LP rat cerebral tissues yielded a single band at the expected weight of corresponding proteins. The expressions of AT1R by western blot was 28.7% lower in the whole brain of 16-week-old LP rats than in NP rats (P<0.001) at the same age. Conversely, an increased expression of AT2R was observed in LP (141.56%) compared to NP offspring (P< 0.05).The data obtained in the present study show by immunostained technique a striking immuno expression of AT2R, POMC and ACTH associated with reduction of AT1R, AVP, MR and GR in hipothalamic-hypophisis axys of 16 wk-old LP rats.

The current study showed a pronounced increase of hypothalamic-pituitary POMC and ACTH expression associated with downregulation of MR and GR immunomarkers in LP. The results suggest lack of control of HHA axys with presumible high corticosterone plasma levels in maternal food-restricted adulthood progenie. These findings are accompanied by reducted diencephalic AT1R and AVP probabily resulting, in decreased kidney water/salt reabsorption and consequently fall of blood pressure as counterregulation phenomenon.

PII-200

Early Catch-Up Growth after Intrauterine Growth Restriction Alters Central Leptin Signal and Energy Homeostasis. Bérengère Coupé, Isabelle Grit, Patricia Parnet. *UMR 1280 Physiology of Nutritional Adaptations, INRA-Nantes University, France.*

Intrauterine growth restriction (IUGR) is closely linked with metabolic diseases, appetite disorders and obesity at adulthood. Leptin, a major adipokine secreted by adipose tissue, circulates in direct proportion to body fat stores, enters the brain and regulates food intake and energy expenditure. Deficient leptin neuronal signalling favours weight gain by affecting central homeostatic circuitry.

The aim of this study was to determine a possible leptin resistance programmed by perinatal nutritional environment and the mechanisms underneath.

Three experimental groups of pups were created from sixteen pregnant Sprague-Dawley rat dams, housed individually and fed either a normal protein diet (20% of protein) for eight of them or an isocaloric low protein diet (8% of protein) for eight of them. At delivery, pups born from restricted mothers or normally fed mothers were adopted randomly by eight control foster mothers (C) to create three experimental groups: CC (n = 4 dams), RC (n = 4 dams) and RR (n = 4 dams) where the first and second letter refers to maternal diet during gestation and lactation, respectively. From the age of 40 days, rats were fed a standard laboratory chow (A04, SAFE, Augy, France) until the end of the experiment.

We demonstrated that five months old IUGR rats develop a decrease of leptin sentivity, characterized by no reduction of food intake following an intraperitoneal injection of leptin. Centrally, this leptin resistance was associated with a slight alteration of hypothalamic JAK2/STAT3 pathway activation in response to leptin, no stimulation of PI3K/AKT, a constitutive stimulation of the AMPK pathway and a high activity of mTOR pathway, revealed by western blot analysis. Fat accumulation in IUGR rats appeared in absence of evidence of body weight change and without low grade inflammation markers in adipose tissue.

The present study reveals that leptin resistance is an early marker of metabolic disorders occurring in aged IUGR rats.

PII-201

Maternal Hypercholesterolemia Correlates with Hypertension, C-Reactive Protein Levels and Number of Bone Marrow-Derived Endothelial Progenitor Cell Colonies in Adult Offspring. Maqsood M. Elahi[1], Felino R. Cagampang[1], Sunil K. Ohri[2], Mark A. Hanson[1]. *[1]Institute of Developmental Sciences, , Academic Unit of Human Development & Health, University of Southampton Faculty of Medicine, Southampton, United Kingdom; [2]Wessex Cardiothoracic Centre, Southampton General Hospital, Southampton, United Kingdom.*

We have shown that long-term maternal high-fat (HF) feeding from weaning through pregnancy and lactation predisposes offspring to hypertension, raised plasma lipids, fatty liver, kidney disorders, raised C-reactive protein (CRP) and reduced numbers of bone marrow derived endothelial progenitor cell (EPC) colonies in offspring. To explore the maternal factors involved, we examined the association between maternal plasma cholesterol levels and various aspects of the offspring phenotype.

Virgin C57BL/6 mice were fed either a high fat diet (HF; fat-45% kcal) or standard chow (C; fat-21% kcal) from weaning through pregnancy and lactation. Weaned female offspring were fed a HF or C diet until adulthood (dam–offspring dietary groups HF-HF; HF-C; C-HF and C-C, respectively). Body weight, blood pressure, plasma cholesterol, C-reactive protein (CRP) and EPC colonies were measured at 24, 28 and 36 weeks post-weaning. All data were expressed as mean ± SEM. Differences between group means were assessed by unpaired Student's *t* test for single comparisons. Spearman's correlation analyses were performed between maternal cholesterol and variables of the offspring phenotype. Values of $P<0.05$ were considered significant.

There was a positive correlation between offspring blood pressure or CRP and maternal cholesterol (0.32 and 0.19; respectively; p<0.0001). The number of EPC colonies in the HF-C and HF-HF were inversely correlated with total maternal cholesterol (P<0.001) and LDL-cholesterol (p<0.001), whereas there was no correlation with maternal HDL-cholesterol. This relationship was not observed in C-HF and C-C offspring. By multivariate analysis, maternal total cholesterol (standard coefficient=-0.530, $P<0.001$) and LDL cholesterol (standard coefficient=-0.417, $P<0.01$) levels remained independent predictors of lower EPC colony numbers in offspring.

Maternal hypercholesterolemia induces hypertension, increased CRP levels and a lower number of EPC colonies in adult offspring. Maternal hypercholesterolemia may thus influence endothelial repair, leading to endothelial dysfunction, hypertension and progression to cardiovascular disease in adult offspring.

PII-202

Gender-Specific Cardiovascular Effects of Prepregnancy Maternal Obesity in Sheep. Jorge P. Figueroa, Jie Zhang, Angela G. Massmann. *Obstetrics and Gynecology, Wake Forest University School of Medicine, NC, USA.*

Pre-pregnancy obesity is associated with increased maternal and newborn morbidity. Importantly, offspring of obese mothers are also at increased risk for developing obesity and hypertension. The aim of the present study was to determine the effects of maternal obesity on the regulation of cardiometabolic function in young adult offspring.

Non-pregnant sheep were allocated to be fed Rumilab™ chow at either 100% of their recommended allowance (C) or ad libitum (MOb) for three months. Sheep were mated after induced estrus to the same ram and maintained on the same diet until weaning of the lamb. Offspring (9-12 mo of age) were chronically instrumented under general anesthesia to place vascular catheters and a femoral artery flowprobe. Blood pressure (BP) and femoral artery flow were continuously recorded and vascular resistance (FVR) was derived from these values. We studied the effects of cumulative iv increasing doses of Ang II and norepinephrine (NE) on blood pressure and vascular reactivity. Glucose handling was studied by IVGTT using a 0.25 mg/Kg bolus. Data are expressed as Mean±SEM and were analyzed by ANOVA.

MOb sheep increased their weight by 40% prior to mating. At delivery, body weight was 93±2.6 vs 114±4.9; Kg in C and MOb respectively, p<0.05. At baseline we found that BP and FVR were significantly elevated only in female offspring of MOb (102±2.1 vs 95±0.8; MAP; mmHg) when compared to those of C *p<0.05. No significant differences in BP were observed in males (97±3.1 vs 97±1.9; MAP; mmHg). The FVR response to cumulative administration of five doses of Ang II (1-1.2 pg/Kg/min; 15 min each) was significantly higher in MOb female offspring but not in male offspring (F=23.5, p<0.05). In contrast, FVR response to cumulative NE was not affected by maternal obesity in either sex. No significant differences in glucose handling were observed.

We found a sex-dependent alteration in blood pressure control and vascular reactivity in offspring of prepregnant obese sheep. Female offspring had a significant elevation in arterial blood pressure and enhanced response to exogenous angiotensin but not norepinephrine. Interestingly a difference in birth weight, albeit small, was also observed only among female offspring. Further studies are needed to establish a mechanism by which MOb alters blood pressure control and particularly the renin angiotensin system and the role of birth weight. HL 68728 and HD 04784.

PII-203

Maternal Obesity and High Nutrient Intake in the Ewe Result in Increased Adiposity of Male Offspring: Possible Impact of Pituitary Leptin Resistance on down Regulating the GH/IGF-1 Axis. Stephen P. Ford[1], Tursunjan Nurmamat[1], Nathan M. Long[1], Peter W. Nathanielsz[2]. *[1]The Center for the Study of Fetal Programming, University of Wyoming, USA; [2]Center for Pregnancy and Newborn Research, University of Texas Health Sciences Center, San Antonio, USA.*

Recently, pituitary leptin receptor (OB-Rb) deficiency has been linked to decreased somatotrope numbers and function, suggesting that leptin may serve a role in growth hormone (GH) secretion. We have shown that maternal obesity during gestation in the ewe results in hyperleptinemia, increased adiposity and insulin resistance in adult male offspring. Our objective was to evaluate the impact of maternal obesity and overnutrition on the Leptin/GH/Insulin like growth factor (IGF)-1 axis of these adult male offspring.

From 60 d before conception through parturition, ewes were fed 100% (control, C) or 150% (obese, OB) of NRC recommendations. Male offspring from OB (n=6) and C (n=6) ewes were maintained together and fed to 100% NRC recommendations until two to three years of age and then subjected to a 12-wk ad libitum feeding challenge before necropsy. A jugular blood sample was collected and plasma frozen at -20°C and pituitary and liver tissue collected, and snap frozen at -80ºC until utilized. Blood levels of IGF-1 were measured via a validated assay (Immulite 1000).

Total visceral fat as a % of live wt was greater ($P<0.01$) for OB offspring than C offspring (10.4±0.8 vs. 7.7±1.0%) The pituitary mRNA expressions of leptin, OB-Rb, and GH, as well as, liver mRNA expression of GH receptor and IGF-1 were determined by quantitative RT-PCR, and protein expression of OB-Rb and GH by Western analysis. While pituitary leptin mRNA expression remained similar, mRNA expression of OB-Rb (2.9±0.4 vs. 4.3±0.5; $P < 0.05$) and GH (2.1±0.3 vs. 3.2±0.2; $P < 0.01$) were lower in OB vs. C offspring. Further, liver mRNA expression of GH receptor (2.7±1.2 vs. 9.2±1.2, $P < 0.01$) and IGF-1 (3.2±1.0 vs 6.7±1.0 $P < 0.01$), as well as plasma concentrations of IGF-1 (327±31 vs. 442±19 ng/ml, $p<0.05$) were reduced in OB vs. C offspring. Pituitary protein expression of OB-Rb (0.38±0.03 vs.0.47±0.03) was lower ($P<0.05$), and GH (5.0±0.2 vs. 5.8±0.5) tended to be lower ($P<0.10$) in OB vs. C offspring.

These data suggest that the increased adiposity and insulin resistance observed in male offspring exposed to maternal obesity and high nutrient intake during gestation may be programmed by reduced leptin stimulation of GH/IGF-1 axis.

PII-204

Maternal Obesity and Nutrient Excess throughout Gestation in the Ewe Increases Appetite and Adiposity of Adult Male Offspring, through Increases in Adipocyte Size and Metabolism. Nathan M. Long[1], Daniel C. Rule[2], Peter W. Nathanielsz[3], Stephen P. Ford[1]. *[1]Center for the Study of Fetal Programming, University of Wyoming, WY, USA; [2]Department of Animal Science, University of Wyoming, WY, USA; [3]Center for Pregnancy and Newborn Research, University of Texas, Health Sciences Center, TX, USA.*

In a recently developed sheep model of maternal obesity, newborn lambs exhibiting a markedly increased % body fat at birth. Further, we recently reported that adult male offspring from these obese overfed ewes developed greater adiposity in response to *ad libitum* feeding than adult offspring from normal weight ewes fed to requirements. We hypothesized that adiposity size and metabolism was altered in male offspring from obese vs normal weight ewes.

Multiparous ewes of similar age and body weight were randomly assigned to a control (C, 100% of NRC) or obese (OB, 150% of NRC) group from 60 days before mating to term, then all ewes were fed to requirements during lactation. After weaning, offspring were maintained together, and fed to requirements for maintenance and growth. At three years of age, male offspring (C=7 and OB=6) were fed a highly palatable *ad libitum* diet with feed intake monitored by Growsafe (Airdrie, Alberta, Canada) for 12 weeks.

During the feeding trial OB males consumed more feed than C males ($P<0.01$; 248±5 vs 192±10 kg). Further, OB males gained more weight ($P<0.01$; 35.6±1.0 vs 29.5±2.7 kg), and became heavier ($P<0.05$, 113±3 vs 104±3 kg) then C males by the end of the feeding trial. At necropsy the weights of the perirenal (PR) and omental (OM) adipose depot were increased ($P<0.05$, 47% and 58% greater than C, respectively) in OB vs C males. Subcutaneous (SC) fat thickness was also 41% greater ($P<0.05$) in OB vs C males. The weight of the mesenteric adipose depot was similar between groups. Adipocyte diameters were greater ($P\leq0.04$) in PR, OM and SC adipose depots in OB vs C males (11, 8 and 7% increase compared to C, respectively). When adipose tissue was incubated for two hrs with C-14 labeled acetate, SC, PR, and OM depots of OB males exhibited greater incorporation ($P<0.05$; 290, 83, and 90% increase compared to C, respectively) of acetate into fatty acids than adipose tissue of C males.

Maternal obesity leads to increased appetite and adiposity that is associated with increased adipocyte diameters and increased rate of fatty acid synthesis in overnourished adult male offspring. NIH INBRE P20RR016474.

PII-205

Consequences of Folate Depletion during Development and High Fat Intake from Weaning on Adiposity, Gene Expression and DNA Methylation in Adult Mice. Jill A. McKay[1], Michiel Adriaens[2], Long Xie[1], Caroline Manus[1], Chris T. Evelo[2], Dianne Ford[1], John C. Mathers[1]. *[1]Human Nutrition Research Centre, Newcastle University, Tyne and Wear, United Kingdom; [2]BiGCaT, Maastricht University, Netherlands.*

The DoHAD hypothesis proposes that nutritional insults *in utero* result in altered programming of offspring, causing increased adulthood disease risk. Previously, we observed that maternal folate depletion during pregnancy pre-disposed mice to be heavier ($p=0.016$) and have heavier organs (liver, $p=0.024$; small intestine, $p=0.036$) in adulthood (unpublished data). Folate depletion may influence DNA methylation at CpG dinucleotides through effects on the supply of the methyl donor S-adenosylmethionine, providing a candidate mechanism to explain developmental programming in this model. We investigated the hypothesis that offspring born to folate depleted mothers may be more susceptible to increased adiposity when fed a high fat diet. Furthermore we investigated the influence of maternal folate depletion and post weaning high fat feeding on genome wide DNA methylation patterns and gene expression.

Female C57BL/J6 mice were assigned randomly to folate-adequate (2mg folic acid/kg) or folate-deplete (0.4mg folic acid/kg) diets four weeks prior to mating and remained on allocated diets during pregnancy and lactation. At weaning, offspring were randomly allocated to a low (LF; 5%) or high fat (HF; 20%) diet, resulting in four treatment groups. Total adiposity was assessed by MRI scanning at three and six months of age. Mice were killed two weeks after MRI scanning at six months. DNA and RNA were extracted simultaneously from ground liver tissue. Hepatic RNA from male mice was hybridized to Affymetrix mouse whole genome arrays (MOA430). Methylated hepatic DNA from male mice was immunoprecipitated then amplified by PCR, before hybridization to Roche NimbleGen Mouse DNA Methylation 3x720K CpG Island Plus RefSeq Promoter Arrays.

Folate depletion during development did not have an effect on adiposity in the offspring. At both three and six months post-weaning high fat feeding was associated with increased adiposity ($p<0.001$). Folate depletion during development caused increased expression of 2088 genes and decreased expression of 1279 genes (fold change of +/-1.2 and $p<0.05$). High fat feeding post weaning increased expression of 1089 genes and caused the down regulation of 569 genes (fold change of +/-1.2 and $p<0.05$).

Further analysis of data generated from these arrays will be presented.

PII-206

Feeding the Epidemic of Childhood Obesity. J. A. Marsh[1], S. W. White[1], N. M. Warrington[1], S. J. Lye[2], G. Davey Smith[3], J. P. Newnham[1], L. J. Palmer[2], C. E. Pennell[1]. *[1]School of Women's and Infants' Health, The University of Western Australia, Western Australia, Australia; [2]Samuel Lunenfeld Research Institute, University of Toronto, Toronto, Canada; [3]School of Social Medicine, University of Bristol, Bristol, United Kingdom.*

Nutrient supply to the fetus is mediated by the placenta and influenced by: maternal pre-pregnancy nutrition stores and metabolism, diet during pregnancy, parental and fetal genetics, and the intrauterine environment. *To investigate the relationships between maternal nutrition, placental size and childhood growth and adiposity.*

Data on 1178 male and 1113 female singleton births from the Western Australian Pregnancy (Raine) Cohort, with nine measures of childhood

height & BMI between birth and age 17, were individually summarized based on size, growth velocity, age at adiposity rebound and peak height velocity. Sex-specific multivariate linear regression was performed including variables: ratio of placental weight:birth weight, maternal pre-pregnancy BMI, gestational weight gain, maternal smoking and duration of breast-feeding.

Height in males was associated with maternal BMI and pregnancy weight gain whereas in females breastfeeding duration played a more significant role. Conversely, there were no gender differences in the relationships between maternal BMI and weight gain and childhood adiposity. Increased maternal BMI and weight gain was associated in males with greater childhood stature ($p<0.02$) and lower growth velocity ($p<0.02$) and in both sexes with greater adiposity during childhood ($p<0.006$) and earlier adiposity rebound ($p<0.003$). Increased duration of breastfeeding was associated in females with greater stature ($p=0.006$), higher growth velocity ($p=0.006$) and later age at peak height velocity ($p=0.01$). Maternal smoking was associated with earlier age of adiposity rebound ($p<0.003$) and greater adiposity ($p<0.006$) in both sexes. The ratio of placental to birth weight was higher in females ($p=3.5x10^{-6}$) and negatively associated with length of gestation ($p<2x10^{-16}$). Childhood growth was not associated with ratio of placental to birth weight after adjustment for length of gestation.

Gender appears to play a role in the relationship between early life events and height during childhood whereas concordant associations for the sexes were observed for measures of adiposity. These data provide evidence for the fetal overnutrition hypothesis and suggest that gender influences on skeletal growth are different from those on adiposity.

PII-207

Effect of High Maternal BMI on Folate Concentrations and Placental DNA Methyltransferase 1 (DNMT-1) Gene Expression in Humans: The PREOBE Follow up Study*. Jole Martino[1,2,3], Sylvain Sebert[1], Mairia Teresa M.T. Segura[2], Iryna Rusanova[2], Maria Cristina M.C. Martinez-Zaldivar[2], Luz Garcia-Valdez[2], Maria Carmen M.C. Padilla[3], Harry H.J. Mcardle[4], Helen Budge[1], Michael M.E. Symonds[1], Cristina Campoy[2]. *[1]University of Nottingham, United Kingdom; [2]University of Granada, Spain; [3]University of Granada, Spain; [4]University of Aberdeen, United Kingdom.*

The importance of maternal methyl donors ,including folate, in DNA methylation and embryogenesis has been demonstrated in animal studies. Maternal obesity has been associated with adverse neonatal outcomes which could be linked to folate deficiency. Therefore, we tested whether maternal BMI can alter folate status and whether that, in turn, modifies the activity of placental DNMT-1 and offspring folate concentrations.

Women were recommended to take folate supplements (0.4mg) during the first three months of gestation. Pregnant women were recruited at 20 weeks of gestation and classified according to their pre-pregnancy BMI into control (BMI>25kg/m2;n=56), overweight (25<BMI<30kg/m2;n=26) and obese (BMI>30kg/m2;n=21) groups. Plasma folate concentrations were analysed in maternal samples taken at 34 weeks of pregnancy and at delivery (38.7 ± 1 gestational weeks), and in cord blood. Placental DNMT-1 gene expression was determined using real-time PCR. Data were analysed according to their parametric distribution with Kruskal-Wallis and 1-way ANOVA.

Although there was no difference in maternal folate intake in early pregnancy as determined by lifestyle questionnaire, overweight mothers had lower folate concentrations at both 34 weeks of pregnancy (C=12.9±0.6; OV=10.3±0.9;(p=0.02)) and at delivery (C=12.8±0.7;OV=9.5±0.9;(p=0.005)), whilst in obese women,plasma folate was only reduced at delivery (OB=8.9±1.2ng/mL (p=0.007)). There were no differences in cord blood folate.Placental mRNA abundance of DNMT-1 was upregulated two fold with overweight (C=0.0115±0.0019;OV=0.021±0.002;(p=0.0004)),and 1.5 fold with obesity (OB=0.0168±0.0029;(p=0.04)) but was not directly associated with folate concentrations.

Despite plasma folate concentrations were lower in women of increased BMI, there was no reduction in their newborns, suggesting enhanced placental folate transport. The extent to which upregulation of placental DNMT-1 may act with altered DNA methylation in the placenta to increase folate supply to the fetus is currently being investigated.

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PII-208

Diet and Physical Activity in Severely Obese Pregnancy: Associations with Gestational Weight Gain and Birthweight. Nor A. Mohd-Shukri, Jennifer Bolton, Shareen Forbes, Fiona C. Denison, Jane E. Norman, Brian R. Walker, Rebecca M. Reynolds. *Tommy's Centre for Maternal and Fetal Health, University of Edinburgh, United Kingdom.*

Maternal obesity is associated with adverse effects for mothers and offspring. We hypothesized that obese women eat more total calories (TC) and do less physical activity (PA) than lean during pregnancy, and that this is associated with excess gestational weight gain (GWG) and increased birthweight (BWT). We aimed to assess this in a longitudinal study of severe obesity ($BMI \geq 40 kg/m^2$) in pregnancy.

125 obese and 70 lean (BMI 43.9(0.3) vs. 22.5(0.2), *P<0.001*) pregnant women were recruited. Ethical approval and written informed consent were obtained. Food intake and PA were assessed at 12-20 and 28-36 weeks gestation using validated questionnaires, and accelerometry in a subgroup (n=28/gp). Serial maternal weight and baby BWT were recorded. Data are mean (SEM).

TC was higher (2669(100) vs. 2405(77) kcal/day, *P<0.05*) in obese than lean in early, but not late pregnancy (2379(83) vs 2492(92) kcal/day, *P=NS*).

Obese reported doing more total PA than lean in early (28.4(1.7) vs. 22.5(1.5) MET-hours/day, *P<0.05*) but not late pregnancy. Obese did more light-intensity but less sports/exercise activity throughout pregnancy (*P<0.05).* Accelerometry showed lower activity scores in obese than lean (187(15) vs 256(23) count/min, *P<0.05*).

GWG was lower in obese than lean (5.7(0.5) vs. 10.9(0.6) kg, *P<0.001*) and associated with TC (*r=0.50, P=0.001*) in lean, but not obese. GWG was not related to PA.

Mean term BWT was similar in both groups. In lean, higher BWT (adjusted for gender and gestation period) was associated with increased protein intake (*r=0.39, P=0.02*), reported total PA (*r=0.35, P=0.02*) and reduced sedentary activity (*r=-0.34, P=0.003*). In obese, increased BWT was associated with higher reported sports/exercise activity (*r=0.24, P=0.03*).

Obese women ate more TC during early but reduced to amounts similar to lean during late pregnancy. Obese also reported higher total PA but was not supported by the quantitative accelerometry measurements.

Obese women had less GWG than lean but this was not associated with TC or PA. In contrast to lean, BWT in obese was not related to maternal nutrition. Contrary to other reports, increased sports/exercise activity was associated with higher BWT.

Nutrition and PA in severely obese pregnancy is poorly understood and more research is required to inform of interventions to optimize BWT and GWG.

PII-209

Embryonic Stem Cells: Modelling Effects of Early Embryo Environment on Developmental Potential. Andy Cox, Tom P. Fleming, Neil Smyth. *School of Biological Sciences, University of Southampton, United Kingdom.*

Altered maternal diet exclusively during preimplantation development induces adaptations in offspring, associated with increased risk of adult onset disease. Changes must therefore occur within the distinct stem cell populations of the early embryo and be maintained through development. Here, mouse ES cells are used to characterize mechanisms involved in the embryo's adaptive responses to maternal diet.

ES cell lines were derived from blastocysts of C57BL/6 mice assigned to either a low protein diet (LPD; 9% casein, n=14), or a control diet (18% casein, n=12) exclusively through preimplantation development. These lines were characterized for karyotype, sex, gene expression, and functionally for proliferation and metabolism at standardized passages. Cell proliferation was assessed by haemocytometer counts at 24h intervals over 96h. The proportion of replicating cells was determined by BrdU incorporation. Metabolic activity was determined at 24h intervals over 96h using an MTT assay.

LPD significantly reduced ES cell derivation (17.4% blastocysts yielding ES cell lines after LPD vs. 41.9% in controls). These ES cells have comparable levels of gene expression related to pluripotency, housekeeping and developmental functions irrespective of diet. There was a strong sex bias towards male ES cell lines (78.1%). LPD affected growth patterns of

ES cells in culture. Cell proliferation rates were slower over the first 48h of culture in ES cells derived from LPD treated blastocysts (P<0.05). These cells however showed increased metabolic activity compared to controls over four days culture, which was significantly higher at 96h (P<0.05).
Lowered ES cell isolation efficiency may indicate a reduced number of pluripotent cells within the early embryo or increased sensitivity of these cells in response to maternal LPD. Dietary-induced changes in proliferation and metabolic potential seen in our ES cell model mimic LPD changes in growth and metabolism identified from *in vivo* studies, authenticating the model. These adaptations may further impact on lineage allocation as differentiation occurs. ES cell lines provide access to post-implantation tissues for mechanistic analysis of fetal responses to poor nutrition underlying the induction of adult onset disease.
Research funded by DTA PG studentship (AC) and BBSRC (BB/F007450/1).

PII-210

Expression of Glucocorticoid Receptor and Hydroxysteroid 11-beta Dehydrogenases in Bovine Preimplantation Embryos. Shuntaro Ikeda, Miki Sugimoto, Shinichi Kume. *Kyoto University, Kyoto, Japan.*
Glucocorticoid (GC) exerts its effects by binding to its nuclear receptor (GR), and the activity of GC is also modulated by hydroxysteroid 11-beta dehydrogenases (HSD11B), which catalyze the reversible conversion of the active and inert form of GC. Two biochemically distinct isoforms of HSD11B exist: HSD11B1 predominantly activates 11-keto-steroids by catalyzing their conversion to GC, whereas HSD11B2 converts GC into its inert form. In pregnant animals, placental HSD11B2 protects the fetus from excess maternal GC. Alterations in the function of the placental barrier result in the exposure of the fetus to high levels of GCs in utero, which has been implicated in the developmental origins of health and disease (DOHaD) paradigm, in which events occurring during embryonic and fetal life, such as the fetal exposure to excess maternal GC programs the metabolic disorders in later life. Questions arise as to the action of GC in preimplantation embryos before the formation of the placenta. In the present study, the expression patterns of GR and two isoforms of HSD11B (HSD11B1 and HSD11B2) in bovine preimplantation embryos were investigated at the mRNA and protein levels.
Bovine preimplantation embryos were produced by in vitro fertilization and embryos at the 1-cell, 2-cell, 8-cell, morula, and blastocyst stages were collected. Reverse transcription-polymerase chain reaction (RT-PCR), immunofluorescence, and western blot analyses were performed on these embryos.
RT-PCR analysis revealed the presence of GR and HSD11B2 transcripts in bovine embryos from the 1-cell to blastocyst stage, while HSD11B1 transcripts were not detected. Immunofluorescence analysis in embryos at the 1-cell, 8-cell, and blastocyst stages showed that GR protein was present predominantly in the cytoplasm of embryos in the absence of GC. Stimulation of blastocysts with GC (cortisol) resulted in the accumulation of GR to the perinuclear region in some cells, but the nuclear translocation of GR was hardly detectable. Western blot analysis using embryos at the 1-cell and blastocyst stages showed the expression of HSD11B2 protein but not HSD11B1 in these embryos.
Bovine preimplantation embryos express GR mRNA and protein, and the activity of GC may be regulated in part by endogenous HSD11B2.

PII-211

Maternal Diet Modulates Embryonic and Placental Development in Diabetic Pregnancies. Claudia Kappen[1], Claudia Kruger[1], XiaoYing Zhang[2], Jacalyn MacGowan[2], J. Michael Salbaum[2]. *[1]Developmental Biology, Pennington Biomedical Research Center, LA, USA; [2]Regulation of Gene Expression, Pennington Biomedical Research Center, LA, USA.*
Maternal diabetes during pregnancy is associated with a higher risk for congenital defects, but progeny also carry a higher risk for disease later in life, such as metabolic syndrome and hypertension. In a mouse model of diabetic pregnancy, we sought to identify gene-environment interactions that account for and may be causative for adverse pregnancy outcomes.
Quantitative morphology and histology, gene expression profiling, in situ hybridization, mutant studies, diet studies, statistical evaluation.
We have shown that diabetes-exposed embryos exhibit intrauterine growth retardation, aggravated by an adverse maternal diet. This diet, which derives more calories from fat and less from proteins -without being deficient in any nutrient category-, also increases the risk for neural tube defects. Our gene expression studies implicate known neural tube defects genes as well as epigenetic mechanisms in adverse embryonic outcomes from diabetic pregnancies.
We also identified placental abnormalities in diabetic pregnancies, in particular altered gene expression and impaired placental growth. This is accompanied by aberrant cell differentiation in embryonic and maternal compartments of the placenta. Again, adverse outcomes are modulated by maternal diet, which has significant effects on gene expression in diabetic placenta, evident as early as midgestation in the mouse.
Maternal diet is an important modulator of pregnancy outcomes in high-risk pregnancies (e.g. those affected by maternal diabetes). Our studies highlight gene-environment interactions that are modulated by maternal diet, and provide functional biomarkers for adverse exposure. The early time points at which abnormalities are evident in the embryo and in the placenta indicate that nutritional programming already impacts very early morphogenetic processes in the formation of those tissues. Aberrant cell differentiation during placentation then precipitates long-lasting alterations in hormonal signaling from the placenta, in nutrient transport, and in embryonic and fetal growth trajectories. One implication of these results for human pregnancies is that efforts to prevent adverse pregnancy outcomes would be expected to be more effective if they target the preconception period and the first trimester.

PII-212

Periconceptional Undernutrition Alters Insulin Signalling in Muscle from Late Gestation Sheep Fetus. Shervi Lie[1], Janna L. Morrison[1], Olivia Wyss[1], Susan E. Ozanne[2], Song Zhang[1], Caroline McMillen[1]. *[1]School of Pharmacy and Medical Sciences, University of South Australia, South Australia, Australia; [2]Institute of Metabolic Science, University of Cambridge, Cambridge, United Kingdom.*
Maternal undernutrition during early gestation in human is associated with an increase risk of insulin resistance and glucose intolerance in adulthood. This study aims to investigate the effect of maternal undernutrition during the periconceptional and early preimplantational period (PCUN and PIUN) on factors regulating insulin signalling in late gestational fetal muscle. We hypothesized that PCUN and PIUN would result in a decrease in gene expression of the insulin receptors A and B (IRA,IRB) and a decrease in the protein abundance of the insulin signalling factors, insulin receptor (IR), insulin receptor substrate 1 (IRS1), PI3K regulatory and catalytic subunits (p85, p110β), Akt1, Akt2, phosphorylated Akt (pAKT), Protein Kinase C zeta (PKCζ) and the insulin dependent glucose transporter 4 (GLUT-4). Control ewes were fed 100% metabolizable energy requirement (MER) from -45d to 6d postconception. Ewes in the PCUN group were fed 70% MER from -45d to 6d and ewes in the PIUN group were fed 70% MER from conception to 6d postconception. Skeletal muscle samples from singleton fetuses were collected at 136-138d gestation. The mRNA expression of IRA and IRB were analysed using Real Time-PCR. The protein expression of IR, IRS1, p85, p110β, Akt1, Akt2, pAkt, PKCζ and GLUT-4 were analyse using western blot analyses.
IRA and IRB mRNA expression were lower in the PCUN group (P=0.005, p=0.009, respectively), however, there was no difference in the IR protein expression between the treatment groups. There was no significant difference in the abundance of IRS1, p85, Akt1, Akt2, pAkt and GLUT-4. The abundance of p110β, however, tended to be lower in the PCUN group only (P=0.065), while, the abundance of PKCζ was decreased both in the PCUN and PIUN (P=0.034).
Periconceptional undernutrition resulted in lower mRNA expression of IRA and IRB, and lower abundance of the insulin signalling factors p110β and PKCζ. In addition, the abundance of PKCζ was also lower in the PIUN group. These findings suggest that there are specific factors which are recruited after exposure to undernutrition during the periconceptional and early preimplantational periods which alter gene and protein expression in the insulin signalling pathway in skeletal muscle before birth.

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PII-213

Adaptations during the Preimplantation Period Are Diet-Specific. Francesca Lock, Stephanie Austin, Tom Fleming, Judith Eckert. *DOHaD / Developmental & Cell Biology, University of Southampton, United Kingdom.*

Dietary challenge fed to mice only during the preimplantation period can cause increases in birth weight, hypertension and abnormal behaviour in offspring[1]. A key event during this period is the formation of the blastocyst. This involves the allocation of cells to the trophectoderm (TE) and inner cell mass (ICM) lineages which give rise to the placenta and embryo proper respectively. Adaptation of these stem cell populations and their relative balance in response to maternal diet have the potential to dictate the future potential of the embryo[2,3]. Here we question whether lineage adaptations are a generalized response to poor nutrition or are diet-specific by feeding different overnutrition diets; high fat (HF) or high protein (HP).

MF1 mice were naturally mated at seven weeks of age. On the morning of conception, dams were given HP, HF or control diets. Diet consumption was isocaloric. At 3.5 and 3.75 dpc mice were sacrificed and embryos collected. Differential labelling was used to show TE and ICM populations by position within the embryo.

HF embryos were further investigated. Immunofluorescence was used to discriminate lineage on a molecular basis and protein synthesis was measured by L-Azidohomoalanine (a methionine analogue) incorporation.

Lineage allocation was altered in response to both HP and HF diets. HP embryos showed a decrease in ICM cell number at 3.75 dpc. HF embryos had a significantly greater number of cells in both lineages compared to controls, and also contained a greater proportion of cells within the ICM at 3.5 dpc. Although ICM cell numbers were increased, fewer cells expressed SOX2, a key pluripotency marker. Protein synthesis remained constant despite the increased total cell number.

We have shown a macronutrient specific response of lineage allocation in the preimplantation embryo. These adaptations may have evolved to establish optimal proportional placental size in anticipation of nutrient availability. Adaptation may however result in poor cellular characteristics as observed in the HF embryo. Inappropriate adaptation early in pregnancy may cause continued progression along an altered growth trajectory, and could be a source of later disease. These results highlight the importance of dietary composition for those planning to conceive.

References

1. Watkins *et al.* (2008) BOR 78: 299-306
2. Wang *et al.* (1990) Reproduction, Fertility and Development 2(6): 683- 691
3. Mitchell *et al.* (2009) BOR 80: 622–630

PII-214

Abnormal Expression of L-Arginine/NO-Related Enzymes in HUVEC Is Associated with Altered Vascular Reactivity of Umbilical Vein from IUGR Placentae. Bernardo J. Krause, Ernesto Muñoz, Catalina P. Prieto, Luis Sobrevia, Paola Casanello. *PRL-CMPL, Division of Obstetrics & Gynaecology, School of Medicine, Pontificia Universidad Católica de Chile, Chile.*

Intrauterine Growth Restriction (IUGR) is associated with fetal hypoxia, lower nitric oxide (NO) synthesis by the endothelial NO synthase (eNOS) and altered placental vascular tone. Arginase-2 (Arg-2) has been described to compete with eNOS for their common substrate L-arginine, leading to vascular dysfunction. We studied the role of Arg-2 in the NOS-mediated vascular effects in IUGR umbilical veins (HUV) and in HUV endothelium (HUVEC) under hypoxia.

Normal (N) and IUGR HUVEC were isolated by collagenase digestion and cultured to confluence at 37ºC (air/CO_2, 95/5); cell cultures were exposed to normoxia (5% O_2) and hypoxia (2% O_2, 24 h) and harvested for further analysis. Arg-2, eNOS and p-eNOS (Ser1177) protein levels were determined by western blot, and arginase activity was quantified by urea formation. HUV rings were dissected from placentae and mounted in a wire myograph to determine vasoactive response to insulin (10^{-12} - 10^{-8} M), in presence or absence of the arginase inhibitor S-(2-boronoethyl)-L-cisteine (BEC 10^{-5} M) and the NOS inhibitor L-nitroarginine (L-NA 10^{-4} M). Responses were expressed as a percentage of KCl-induced contraction (%Kmax).

In IUGR HUV, NOS-dependent relaxation was decreased (0.1 ± 2.5%Kmax) compared with N HUV (37.6 ± 3.8%Kmax). Arginase inhibition increased NOS-dependent relaxation in both IUGR (18.1 ± 2.5%Kmax) and N vessels (57.5 ±3.1%Kmax). N HUVEC exposed to hypoxia increased Arg-2 protein levels and activity, and reduced p-eNOS/eNOS levels. In normoxia, Arg-2 levels and activity were higher in IUGR than N HUVEC, whilst there were lower levels of eNOS and p-eNOS. Interestingly, hypoxia had no effects on Arg-2, eNOS and p-eNOS protein levels in IUGR HUVEC.

This data shows that Arg-2 has a role as vascular modulator in placental vessels, and its overexpression could be implicated in the reduced production of NO in N HUVEC under hypoxia. Furthermore, IUGR HUVEC present a hypoxic phenotype even after being cultured in presence of normal or high levels of oxygen suggesting an altered cellular programming.

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PII-215

Maternal Supraphysiological Hypercholesterolemia Leads to Reduced Endothelium-Dependent Vasodilation of Umbilical Vein and Increased L-Arginine Transport in HUVEC. Andrea Leiva, Enrique Guzman-Gutierrez, Fernando Abarzua, Paola Casanello, Luis Sobrevia. *Cellular and Molecular Physiology Laboratory (CMPL) & Perinatology Research Laboratory (PRL), Division of Obstetrics and Gynecology, Pontificia Universidad Catolica de Chile, Chile.*

Maternal physiological hypercholesterolemia (MPH) occurs in pregnancy assuring fetal growth and development. However, when MPH goes over this physiological adaptation, maternal supraphysiological hypercholesterolemia (MSPH), a condition reported to generate aortic atherosclerosis in the fetus and children. **The objetive was** to estimate MSPH incidence in Chilean population and the effect of this condition in placental endothelial function.

MSPH incidence was estimated considering a cut-point >280 mg/dl for maternal blood cholesterol in the third trimester of pregnancy in a population of pregnant women (n=12) of Hospital Clínico UC (Santiago de Chile). Umbilical vein rings from women with MPH and MSPH were dissected and mounted in a wire-myograph and endothelium dependent (calcitonin gene-related peptide, CGRP, 10^{-10}-10^{-7} M) or independent (sodium nitroprusside, SNP, 10^{-5} M) vasodilatation was determined in KCl-preconstricted vessels. Responses were expressed as percentage of relaxation relative to KCl-induced contraction (%K_{max}) and adjusted to concentration-response curves. L-Arginine transport (30-500 µM, 3 µCi/ml, 37ºC, 1 minute) was measured in primary cultures of human umbilical vein endothelial cells (HUVEC).

A 30% of MSPH incidence was identified in this study. MSPH was associated with reduced CGRP-induced relaxation (~82%) with a half-maximal (IC_{50}) effect reached at 3.2 ± 0.26 nM for MSPH compared with 0.15 ± 0.002 nM for MPH. However, SNP-induced vasodilatation was unaltered by MSPH. Parallel results show increased L-arginine transport in HUVEC from MSPH associated with higher maximal velocity (V_{max} = 0.65 ± 0.05 pmol/µg protein/minute) and apparent Michaelis-Menten constant (K_m = 63 ± 15 µM) compared with cells from MPH (V_{max} = 0.16 ± 0.01 pmol/ug protein/minute, K_m = 14 ± 8 µM).

This is the first description of the high incidence of MSPH in Chilean population. The consequences of this condition relate with abnormal placental vascular function, an effect that could be primary in adult cardiovascular disease as a result of MSPH-associated fetal programming.

PII-216

Development of an Ovine Surgical Model of Uterine Space Restriction: Uterine Anomalies and Multi-Fetal Gestations on Placental Growth and Uterine Vascular Adaptations. Ronald R. Magness, Katie M. Meyer, Mary Y. Sun, Jayanth Ramadoss, Pamela J. Kling. *Depts of Ob/Gyn, Pediatrics, Animal Sciences, University of Wisconsin-Madison, WI, USA.*

Intrauterine Growth Restriction (IUGR) is observed in conditions with limitations in uterine space (e.g. uterine anomalies and multi-fetal gestations). IUGR is associated with reduced fetal weight, organ growth, and the development of a spectrum of adult on-set diseases.

We examined the interaction of uterine anomalies and multi-fetal gestations (Meyer *et al.*, BOR 2010). This surgical model creates a unicornuate/didelphic uterus to study space restriction and local uterine artery (UA)

adaptations. Prior to conception (>60d), all inter-corneal connections were severed, one horn was ligated/resection leaving a gravid horn vasculature locally exposed to uteroplacental factors and the other non-gravid horn vasculature exposed only to systemic factors. Uterine Blood Flows, UA eNOS/NO, placentas and fetuses were studied on Gestational Day (GD) 120 and 130 (Term=147d).

Unilateral surgery decreased placentome numbers in singleton and twin pregnancies (25% & 50%), but not triplets. Fetuses were categorized as uterine space restricted (USR; unilateral twin, and both groups of triplets) had 51% fewer placentomes/fetus and 31% reduction in placentomal weight/fetus vs non-restricted group (NSR; unilateral singleton control singleton & control twin). By GD 130, USR fetuses exhibited decreased weight, smaller crown-rump, abdominal girth, thoracic girth, fetal heart, kidney, liver, spleen, and thymus weights; asymmetric brain sparing IUGR was observed. UBF, UA shear stress (SS), eNOS/NO and vascular remodeling were substantially greater in gravid vs nongravid horn of the unilateral model. This maintained oxygen delivery/extraction independent of measures of placental efficiency.

This model allows for examination of interactive effects of uterine space restriction induced IUGR on uterine and placental adaptation and fetal organ growth. We specifically noted that local, but not systemic, placental factors during gestation are responsible for elevations in UBF, UA SS and remodeling to maintain fetal oxygenation. ***NIH* HL49210, HD38843, HL87144**

PII-217

Maternal Obesity Is Associated with Altered Placental Expression and DNA Methylation of Key Genes in Fetal Growth in Human Pregnancy during the First Trimester, but Not at Term. James R. O'Reilly[1], Amanda J. Drake[1], Rebecca L. Jones[2], Jane E. Norman[1], Jonathan R. Seckl[1], Rebecca M. Reynolds[1]. *[1]Tommys Centre for Maternal and Fetal Health, University of Edinburgh, United Kingdom; [2]Maternal and Fetal Health Research Centre, University of Manchester, United Kingdom.*

Maternal obesity during pregnancy is associated with effects on fetal growth and development. Underlying mechanisms are unknown. We hypothesized that maternal obesity would be associated with altered expression and DNA methylation of key genes involved in placental growth and function.

We aimed to

i) identify a suitable control gene for studying effects of obesity on gene expression in human first-trimester (F) and term (T) placental tissues;

ii) investigate the relationship between BMI and gene expression and DNA methylation in F and T placental tissues.

Nineteen F samples were obtained from women (BMI 18.7-40.4 kg/m²) undergoing surgical termination of pregnancy at 10 weeks (± 11 days; viable intrauterine pregnancy confirmed by ultrasound) gestation. Forty T samples matched for age, gestation, gender and birthweight were collected from lean (BMI<25 kg/m²) and obese (BMI>30 kg/m²) women undergoing elective caesarean section. Ethical approval and written informed consent were obtained.

mRNA expression of six candidate control genes, insulin-like growth factor 2 (*IGF2*), glucocorticoid receptor (*GR*) and 11 beta-hydroxysteroid dehydrogenase type 2 (*11BHSD2*) was measured by real time PCR. Methylation of a differentially methylated region (DMR0) of IGF2 and exon 1 of GR was measured by pyrosequencing.

TBP was identified as a suitable control gene; expression was not related to maternal BMI (r^2=0.004, p=0.92) or offspring gender (r^2=0.04, p=0.23). In F, maternal BMI was positively correlated with expression of *11BHSD2* (r^2=0.54, p<0.005), *GR* (r^2=0.33, p<0.05) and *IGF2* (r^2=0.25, p<0.05). Methylation of *IGF2* DMR0 positively correlated with maternal BMI (r^2=0.57, p<0.005). *GR* methylation was unrelated to BMI. There were no significant differences in gene expression or methylation between lean and obese in T placenta.

Maternal BMI is associated with expression and methylation of key genes in fetal growth and glucocorticoid pathways in first trimester but not term placental tissue. Early pregnancy may be a key period for the adverse effects of maternal obesity on feto-placental growth. Early pregnancy tissues may be more useful for studying the effects of maternal obesity on pregnancy than term tissues.

PII-218

High First Trimester Weight Gain Is Associated with Placental Thinness and Low Omega-3 Fatty Acid Levels in Male Offspring. Perrie F. O'Tierney[1], Melanie Gillingham[2], Kent Thornburg[1,3]. *[1]Heart Research Center, Oregon Health & Science University, USA; [2]Mol. & Med. Genetics, Oregon Health & Science University, USA; [3]Medicine (Cardiovasc. Med.), Oregon Health & Science University, USA.*

The fetus relies on maternal nutrient supply and placental delivery for its supply of omega-3 polyunsaturated fatty acids (n-3 PUFA) which are critical for development. We aimed to determine how maternal weight gain affects placental size and cord blood n-3 PUFA levels at birth in male and female offspring.

40 women were recruited upon admission to OHSU Labor & Delivery for caesarean section. Fasting maternal and umbilical venous blood samples were collected. Fatty acid profiles were quantified using gas chromatography-mass spectrometry in plasma. First trimester weight gain (kg) was calculated based on early second trimester and prepregnancy weights. The relationship between weight gain, placental size and plasma n-3 LCPUFA levels (μM) was determined.

First trimester weight gain was negatively correlated with maternal n-3 PUFA (R=-0.80, p<0.05) and this was not affected by fetal sex. High maternal weight gain in the first trimester was negatively associated with placental thickness (R= -0.69, p<0.05) and cord n-3 PUFA levels (R=-0.70, p<0.05) in male, but not female, offspring.

1) Maternal omega-3 PUFA levels at delivery are negatively related to first trimester weight gain in pregnancies carrying either males or females. 2) First trimester weight gain is associated with placental thinness and low omega-3 fatty acid levels in male, but not female, cord blood. We speculate that male fetuses respond to maternal weight gain early in pregnancy by building a thinner placenta to reserve energy for somatic growth. However, when maternal n-3 PUFA levels are low, a thin placenta may not provide the fetus with sufficient fatty acids necessary for high male growth rates.

PII-219

Cholestatic Pregnancy Increases Susceptibility of Offspring to Metabolic Disease. A Role for Placenta? Georgia Papacleovoulou[1], Shadi Abu-Hayyeh[1], Bryn Owen[1], Alexandra Milona[1], Alex Knisely[2], Eugene Jansen[3], Catherine Williamson[1]. *[1]Institute of Reproductive and Developmental Biology, Imperial College london, Maternal and Fetal Disease Group, United Kingdom; [2]Liver Studies, King's College Hospital, United Kingdom; [3]Health Protection Research, Public Health and the Environment, Netherlands.*

Intrahepatic cholestasis of pregnancy is a liver disease of pregnancy that presents with increased maternal bile acid serum levels. It affects 0.7% of women in UK. The mother also has raised LDL-cholesterol and triglycerides. It can cause fetal distress and spontaneous intrauterine death. We aimed to investigate whether cholestatic pregnancy has long-term effects on the subsequent health of the offspring.

We used a mouse model that was fed a normal chow (NC) diet supplemented with 0.5% cholic acid (CA) during pregnancy. *In vivo* and *in vitro* physiological, biochemical, histological and molecular studies were performed in five-month-old male and female offspring following administration of a NC or a western diet (WD) for six weeks.

Cholestatic pregnancy resulted in an obese and diabetic phenotype in the female offspring fed a WD. Specifically, they had an increased BMI and impaired glucose tolerance. Moreover, lipid measurements showed increased serum total cholesterol and LDL-cholesterol and increased hepatic free fatty acid levels. Hepatic gene expression profiles revealed increased mRNA expression of lipid sensors (e.g. Lxr-α, Ppar-α and Ppar-γ).

To dissect the underlying mechanisms through which cholestatic pregnancy programs this phenotype we also assessed the effects of CA feeding on the fetoplacental unit. Fetuses collected on day 18 of pregnancy had increased hepatic cholesterol and fatty acid biosynthetic pathways (Srebp2/Hmgcr and Srebp1c/Fas) as well as increased levels of hepatic free fatty acids and total cholesterol, with no changes in serum levels. Intriguingly, cholestatic placentas were characterized by increased fat deposition, raised cholesterol and cholesteryl esters accompanied by increased expression of lipogenic pathways and decreased expression of fatty acid transport pathways.

Collectively, these data suggest that altered lipid pathways in the fetuses as

a result of intrauterine exposure to CA increase susceptibility of offspring to metabolic disease. Interestingly, this effect appears to be gender specific. This is the first evidence of cholestatic pregnancy programming subsequent metabolic disease in the offspring.

PII-220

A Retrospective Placental Analysis of Newborn Infants Referred for Neurological Consultation: Early Roots of Neurodevelopmental Risk?
Michael Presta[1], Romaine Schubert[1], Samantha VanHorn[1,2], Pramod Narula[1], Sanford Lederman[1], Carolyn Salafia[1,3]. *[1]New York Methodist Hospital, USA; [2]Institute for Basic Research, USA; [3]Placental Analytics, LLC, USA.*
The correlation of placental pathology and poor neurological exam scores in newborns has not been studied. Specific lesions (e.g. fetal thrombotic vasculopathy, chorioamnionitis) have demonstrated associations with later poor outcomes. The objective of this study was to explore correlations of newborn neurologic exam score with placental shape and gross and histologic placental lesions.
All newborn neurological consults at New York Methodist Hospital from 2007-2009 period (N=59) were reviewed retrospectively. Three factors (gestational and early newborn medical history, general physical and neurological exam, and early CNS imaging) were scored as 1 (normal)- 3 (greatest likelihood of long-term neurodevelopmental impairment). The summed neurological score ranged from three to nine. Placental pathology reports were extracted for variables related to placental shape, infection, infarct and fetal vascular pathology (among others), by a single reviewer blinded to clinical data, and analyses were adjusted for birthweight. Regression considered $p<0.05$ significant.
As the neurological score increased, the umbilical cord insertion was more eccentric ($p=0.01$), and both the number of infarcts in the placenta ($p<0.0001$) and the incidence of avascular villi (marking small fetoplacental vessels obliterated at least 5-7 days before birth, $p=0.02$) increased. Placental thickness tended to decrease as the neurological exam score increased ($p=.05$); chorionic surface dimensions showed no correlation ($p>0.030$). Chronic villitis and histological evidence of acute intraamniotic infection were not correlated with abnormal neurological score.
Abnormal newborn neurological scores are associated with placental infarcts (a maternal vascular lesion) and avascular villi, the most common type of fetal vascular pathology in our data set, independent of birth weight. The lack of correlation of other types of fetal vascular pathology may have been due to their low incidence in this population. Eccentric cord insertion indicating early asymmetry in placental growth, and a trend toward reduced placental thickness, indicating reduced chorionic arborization, was also shown, suggesting that poor newborn neurologic function and future risk has its roots remote to delivery.

PII-221

Why Is There Variety in Placental Shape: Early Influences vs Trophotropism?
C. Salafia[1,2], M. Yampolsky[3], N. Schwartz[4], O. Shlakhter[5], D. Mandel[6]. *[1]Placental Analytics, LLC, USA; [2]Institute for Basic Research, USA; [3]Mathematics, University of Toronto, Canada; [4]Rotman School of Management, Unversity of Toronto, Canada; [5]Obstetrics and Gynecology, University of Pennsylvania, USA.*
Our modeling of placental vascular growth suggests that many common abnormalities of placental shape, such as marginality of umbilical cord insertion, or multi-lobate shape are results of processes which influence early placental growth, and not trophotropism. We test this hypothesis by considering the data gathered from 3D scans of the placentas in the end of the first trimester of pregnancy, followed up by a set of placental measurements after delivery.
During the nuchal translucency exam, the trans-abdominal probe (GE Voluson E8) was used to obtain a 3D volume sweep of the placenta, with measurement of maternal surface diameters, cord location, and placental thickness. Post delivery, the placenta was weighed, and its surface was digitally photographed. A total of 93 placentas were thus measured. Cross-sections of the surface and measurements of placental disk thickness were recorded in 50 of the cases. The modeling of placental vascular growth was carried out using a stochastic growth model based on Diffusion Limited Aggregation (DLA), employing the software package *DLA-3D-Placenta,* which we have developed.
1. Strong and significant correlations are observed between the umbilical cord marginality at 11-14 weeks and at delivery.
2. Placental thickness is correlated with cord marginality when both measurements are taken both at 11-14 weeks and at delivery.
3. A marginal cord insertion at 11-14 weeks, as well as at delivery, strongly correlates with lowered chorionic vascular density at delivery.
Consistent with our modeling, placentas at 11-14 weeks are typically not round, and the early non-roundness does not influence the regularity of the shape at delivery. In our previous work (Yampolsky *et al.*, 2008) we have shown that bi-lobate placentas have normal vascular efficiency. Our empirical modeling suggests that bi-lobate shape is associated with irregularly shaped early vasculogenic zone; this is consistent with increase in number of bi-lobate placentas in IVF (c.f., Jauniaux *et al.*, 1990). Our findings support the conclusions of empirical DLA modeling: common irregularities of placental surface shape such as marginal umbilical cord insertion and multi-lobate shape result from early influences, and not trophotropism.

PII-222

Childhood Growth; a Predictor of Serum Uric Acid in Adult Age in a High Birth Weight Population.
Ingibjorg Gunnarsdottir[1], Thorhallur I. Halldorsson[1], Vilmundur Gudnason[2,3], Thor Aspelund[2,3], Inga Thorsdottir[1]. *[1]Unit for Nutrition Research, University of Iceland & Landspitali National University Hospital, Iceland; [2]Icelandic Heart Association, Iceland; [3]University of Iceland, Iceland.*
Serum uric acid is a marker of number of adverse health outcomes. Low birth weight infants have been observed to have elevated uric acid levels during childhood and overweight at adolescent age has been associated with raised levels at adult age. The aim of this study was to explore the association between growth and serum uric acid at adult age.
A cohort of 2120 subjects born in Reykjavik Iceland 1921-1935, recruited into a longitudional study and medically examined 1967-1991. Information on birth weight and growth between eight to 13 years was collected from National archives. Association between growth and uric acid was examine using multiavariate linear regression adjusting for birth year and age at follow-up. Elevated uric acid was defined as values above the 90th percentile for males (417 µmol/L) and females (339 µmol/L).
The mean birth weight was 3.74kg and mean age at follow-up was 51 years (standard deviation (SD):6 years). Mean uric acid levels at follow-up were 336 (SD: 66) µmol/L for males and 260 (SD:60) for females. Birth weight was not associated serum uric acid at adult age for males with adjusted coefficient of 1 µmol/L increase in serum uric acid per kg birth weight (95:CI: -6,8). A non-significant trend was, however, observed for females -5 µmol/L per kg birth weight (95%CI:-12,1). Compared to normal weight subjects, being overweight at some point between eight to 13 years was associated with 29 µmol/L increase in serum uric acid at adult age (95%CI:11,47) for males and 12 µmol/L (95%CI:0,25) for females. The corresponding odds ratios for elevated serum uric acid were 2.5 (95%CI:1.2, 5.1) and 1.8 (95%CI:0.9,3,5) for males and females, respectively. Furthermore there was a modest positive association between linear height and serum uric acid. The strength of the association increased with age and at 13 years, males in the highest compared with the lowest tertile in the height distribution had 22 µmol/L (95%CI:10,33) higher serum uric acid levels. The corresponding increase for females was 13 µmol/L (95%CI:3,24).
Weight and height between eight to 13 years predicts serum uric acid at adult age. No tracking was observed for birth weight. In general the observed associations were stronger in males compared to females.

PII-223

Are Formula Fed Infants Overfed in Early Infancy?
Shelly N. Hester[1], Deborah S. Hustead[2], Amy D. Mackey[2], Atul Singhal[3], Barbara J. Marriage[2]. *[1]Abbott Nutrition, Champaign-Urbana, IL, USA; [2]Abbott Nutrition, Columbus, OH, USA; [3]Childhood Nutrition Research Center, Institute of Child Health, London, United Kingdom.*
Faster weight gain in infancy has been suggested to contribute to a greater risk of later obesity in formula fed (FF) compared to breast fed (BF) infants possibly due to higher energy content of formula compared to breast milk (BM) and higher protein intake in infants FF compared to BF, especially during the first weeks of lactation. However, few studies have focused on the nutritional composition of BM in the critical window in the first weeks

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after birth. We therefore conducted a systematic review of the literature and meta-analysis of the published data to assess the energy and protein content of BM and volume of milk intake in BF and FF infants in the first two weeks.

Medline databases were used to conduct a systematic review of the literature. All studies from healthy, term, singleton infants that reported values during the first month of life were included. Meta-analyses were performed using Comprehensive Meta-Analysis software, Version 2, Biostat, Inc. (New Jersey USA). Data are presented as MEAN ± SEM.

BM energy was 53.6±2.5 kcal/100mL in colostrum (age 1-5d), 57.7±4.2 kcal/100mL in transitional milk (6-14d), and 65.2±1.1 kcal/100mL in mature milk (>14d) compared to 67 kcal/100mL in formula. The protein concentration of BM was 2.5 ± 0.2 g/100mL in colostrum, 1.7±0.1 g/100mL in transitional milk, and 1.3±0.1 g/100mL in mature milk compared to 1.4 g/100mL commonly found in formula. The volume of formula consumed was substantially higher than the volume of BM on all days analyzed during the first two weeks. For instance, milk intake on the first day of life was 21.5±4.2 mL in BF infants, compared to 170.5±55.8 mL in FF. By day 14, milk intake increased to 674.6±29.0 mL/day in infants BF, compared to 765.5±21.8 mL/day in FF infants. Due to the greater volume of milk intake, protein intake on the first day was 2.4 g in FF infants compared to only 0.5 g in BF infants (a 4.8 fold difference).

Compared to BF, FF infants have a substantially higher energy and protein intake in the first two weeks of life which could predispose them to faster weight gain, and as shown previously, program a greater risk of long-term obesity.

PII-224

Young Adult Metabolic Clustering Associated with Fatness from 12 Months Old. Rae-Chi Huang[1], Trevor A. Mori[1], Chi Le Ha[1], Sally Burrows[1], Wendy H. Oddy[2], Carly Herbison[2], Lawrence J. Beilin[1]. *[1]University of Western Australia, Australia; [2]Telethon Institute for Child Health Research, Australia.*

Low birthweight has been associated with subsequent cardiovascular risk and specifically with central visceral adiposity. Our aim was investigate the associations between metabolic clusters in adult life with body fat distribution throughout life.

One thousand and fifty three adolescents aged 17 years had anthropometry, fasting insulin, glucose, lipids and sphygmomanometer blood pressure readings measured. Two-step cluster analysis identified children at high metabolic risk without using arbitrary definitions of the metabolic syndrome. The high and low risk groups were compared with regards to fat distribution from nine time-points through infancy and childhood.

The metabolic cluster at age 17 years comprised 19% of the males and 24% of the females. Compared to males in the low risk cluster those in the high risk metabolic cluster had higher waist circumference 96.6cm (93.5, 99.8) vs 77.8cm (77.1, 78.4); triglycerides 1.76mmol/L (1.58, 1.94) vs 0.92mmol/L (0.88, 0.95); insulin 16.56pmol/L (14.52, 18.60) vs 7.07pmol/L (6.66, 7.48); systolic blood pressure (SBP) 125.5mmHg (123.5, 127.6) vs 116.6 mmHg (115.8, 117.4); (all $p<0.001$). Compared to females in the low risk cluster, those in the metabolic cluster had higher waist circumference 93.2cm (90.5, 95.9) vs 77.8cm (77.1, 78.4); triglycerides 1.45mmol/L (1.30, 1.60) vs 0.94mmol/L (0.90, 0.98); insulin 20.65pmol/L (15.35,25.96) vs 7.73pmol/L (7.32,8.15); (all $p<0.001$). Compared to the low risk cluster, the high risk cluster was associated with higher birthweight in girls (3406g (3300, 3513) vs 3260g (3203, 3318)) ($p=0.026$). The high risk cluster also associated with greater skinfold thickness in girls from 12 months of age and in boys from three years onwards. 12 month infant girls in the metabolic cluster had higher subscapular skinfold thickness (12.4mm (11.3, 13.6) vs 8.5mm (8.2, 8.9)) ($p<0.001$)); suprailiac skinfold thickness (8.4mm (7.5, 9.3) vs 6.0mm (5.8, 6.2)) ($p<0.001$) and chest circumference (48.0cm (47.6, 48.4) vs 47.4cm (47.2, 47.6)) ($p=0.022$). SBP differences between clusters were detected at age one in girls and age five in boys.

Beyond BMI, changes in fat distribution predict metabolic risk from early life. It also suggests that large for gestational age babies who become at risk metabolically may be more common in females and that traditional in-utero fetal programming more common in males.

PII-225

Neonatal Growth Rate after Undernourishment *In Utero* Positively Correlates with Chronic Inflammatory Reactions in the White Adipose Tissues of Adult Mice, as a Risk of Developing Obesity. Hiroaki Itoh, Kohmura Kobayashi Yukiko, Keiko Muramatsu, Toshiyuki Uchida, Kazunao Suzuki, Naohiro Kanayama. *Department of Obstetrics and Gynecology and Hamamatsu Birth Cohort for Mothers and Children (HBC) Study Team, Hamamatsu University School of Medicine, Japan.*

The neonatal catch-up growth after undernourishment *in utero* is reported to be a risk factor of adult obesity. Recently, it has been demonstrated that the chronic inflammation in the white adipose tissues (WAT) plays a pivotal role in the development of obesity and associated metabolic disorders. In this study, we hypothesized that high neonatal growth rate after undernourishment *in utero* accelerates chronic inflammation in adult WAT and constitutes a risk of obesity under western life style. To prove the hypothesis, we developed a mouse animal model of undernourishment *in utero* and compared neonatal growth rate with the expression of various inflammatory parameters in adult WAT under a high fat diet.

Maternal 30% caloric restriction was apply to C57/BL6 pregnant mice in the latter half of pregnant period. Neonatal growth rate (NGR) was estimated by the formula of [(body weight of 18 days of age [g]) – (body weight of 12 days of age [g])]/(body weight of 12 days of age [g]). A high fat diet (HFD; 60% fat) was applied to undernourished (UN) and normally nourished (NN) offspring, from nine to 17 weeks of age, followed by sampling of subcutaneous WAT. The immunohistochemical detection rate of macrophage specific F4/80 were compared with NGR in NN or UN offspring. The gene expression of monocyte chemoattractant protein-1 (MCP-1) or TNF alpha was measured by quantitative RT-PCR and compared with NGR in NN or UN offspring.

The mean birth weight of UN offspring was significantly lower than that of NN offspring (89%, $p<0.001$). The weight of subcutaneous WAT at 17 wks positively correlated with NGR in UN, but not NN, offspring ($r=0.64$, $p<0.001$). The positive rate of macrophage specific F4/80 immunostaining in WAT positively correlated with NGR in UN, but not NN, offspring ($r=0.39$, $p<0.05$). The gene expression of MCP-1 ($r=0.50$, $p<0.05$) or TNF alpha ($r=0.55$, $p<0.05$) in WAT positively correlated with NGR in UN, but not NN, offspring.

It is suggested that high growth rate of the neonates with undernourishment *in utero* accelerates chronic inflammation in the adult WAT in response to a HFD and contributes, at least partly, to the deterioration of obesity.

PII-226

The Association between Infant Nutrition and Growth, and Pre-Pubertal Body Composition in Urban South African Children. Juliana Kagura, Shane A. Norris, Alison Feeley. *Paediatrics, University of Witwatersrand, Gauteng, South Africa.*

To investigate the association of infant nutrition and growth with pre-pubertal body composition.

This study comprised of 140 children who had infant nutrition and growth measures plus DXA-derived body composition measurements by 10 years of age. They were selected from the Bone health sub-study of the Birth-Twenty cohort comprising of children born in Soweto-Johannesburg in 1990. Infant breastfeeding data was collected during the first 12 months, an non-quantified FFQ was used for dietary patterns at age one year and from this we computed a Dietary Diversity Score (DDS) and Food Variety Score (FVS), house-hold socio-economic status was determined, anthropometry at ages one and two years were used to determine stunting, underweight and wasting prevalence using the WHO growth standards, and at 9/10 years we measured height, weight, and DXA-derived body composition (fat and lean-tissue mass). Regression models were used to determine associations between early factors and pre-pubertal body composition.

All the associations between infant nutrition variables (breastfeeding, bottle feeding, introduction of solids, DDS and FVS) and pre-pubertal body composition were statistically non-significant. A significant increase in birth weight predicted an increase in lean mass ($\beta=0.20$, CI=0.01-0.03, $p=0.007$). Infants who were stunted by age two had significantly lower fat mass ($\beta=-0.37$, CI=-0.64-0.10, $p=0.008$) and lean mass ($\beta=-0.12$, CI=-0.21-0.02, $p=0.018$) at age 9/10 years. Being underweight at age two years predicted lower fat mass in the multivariate model ($\beta=-0.01$, CI= -0.20

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to 0.01, p=0.023). There were no statistically significant relationships between underweight at age 1, wasting at age one and two years with fat and lean mass.
In this study, infant nutrition had no significant influence on subsequent body composition. The relationship between birth weight and lean mass at age 9/10 years is of importance in respect to the link between low birth weight and chronic disease risk, in particular type-2 diabetes. From this study, stunting in infancy did not show any tendency towards higher fat mass at age 9/10 years but rather reduced fat and lean mass. There is need to assess these associations during adolescence to investigate whether adolescence modifies these associations.

PII-227

Low Birth Weight Children Who Put on Weight Become Thin-Fat Adults. S. V. Kasture[1], A. Pande[1], S. Joshi[1], H. Lubree[1], S. Hardikar[1], A. Pandit[2], C. H.D. Fall[3], C. S. Yajnik[1]. *[1]Diabetes Unit, KEM Hospital Research Centre, Pune, Maharashtra, India; [2]Department of Paediatrics, KEM Hospital Research Centre, Pune, Maharashtra, India; [3]MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, United Kingdom.*
Body composition in adulthood is determined by pre natal and post natal growth. This study aims at exploring the contribution of birth weight and subsequent growth to adiposity and lean mass at 21y in the Pune Children Study (PCS).
We studied 351 young adults (21 y) from the PCS, which is a birth cohort of urban children born in KEM Hospital, Pune. Six pregnant girls were excluded from this measurement. Body composition (fat mass, lean mass, bone mineral content) was assessed using dual energy X-ray absorptiometry (DXA) by Lunar Prodigy densitometer. Birth weight was available in the database.
Participants were 57kg, 166cm with a mean BMI of 22kg/m2. Nineteen percent were overweight (BMI > 25-30 kg/m2) and 3% were obese (BMI > 30 kg/m2). Thirty two percent were centrally obese (WHR > 0.90 for males and > 0.80 for females). On DXA measurements 46% were adipose (body fat > 25% for males and > 35% for females).
On linear regression analysis, higher birth weight was associated with higher lean mass and bone mineral content (BMC), in both males and females, and higher fat mass only in females ($p < 0.01$ for all). Higher current weight was associated with all of the body composition outcomes in both males and females ($p < 0.01$, all).
In multiple linear regression analysis with birth weight and current weight together (combined Lucas model), lower birth weight and higher current weight predicted higher fat mass and fat percent, but lower lean percentage ($p<0.01$, all). Only higher current weight was associated with higher BMC ($p < 0.01$). Results were similar in males and females. The interaction term (birth weight x current weight) was not significant for any outcomes.
Children who were lower birth weight and had higher current weight are thin-fat individuals. This phenotype is associated with a number of non-communicable diseases. Improving fetal growth and avoiding excess post-natal nutrition may help reduce the epidemic of non-communicable disease in India.

PII-228

Adiponectin Decreases Risk for Insulin Resistance at Age 71 in Men of Normal BMI and Birth Weight. Amal Khanolkar[1,4], Liisa Byberg[2], George Ploubidis[3], Ilona Koupil[1]. *[1]Centre for Health Equity Studies (CHESS), Karolinska Institutet/Stockholm University, Sweden; [2]Uppsala Clinical Research Center and Department of Surgical Sciences, Uppsala University, Sweden; [3]Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, United Kingdom; [4]Institute for Environmental Medicine, Karolinska Institutet, Sweden.*
To investigate the role of adiponectin in the association between birth weight (BW) and insulin resistance in elderly men.
We studied 727 men born 1920-24 and resident in Uppsala, Sweden, in 1970, who were part of the ULSAM cohort study with more than 38 years follow-up. Information on serum adiponectin, clinical measures of insulin resistance at age 71, BW from medical records, socioeconomic data from routine registers and lifestyle data from questionnaires were available. BW was categorized as low or normal by generating population-specific cut-offs according to Wilcox's hypothesis.
Main outcome measure was insulin resistance measured as a latent variable comprising two indicators: insulin sensitivity index and homeostatic model assessment (HOMA) measured when the participants attended the investigation in 1990. Serum adiponectin was measured by routine laboratory analysis at the same investigation along with other blood parameters. BMI at age 71 was derived from anthropometric measurements. Participants answered questionnaires on lifestyle-related activities including physical activity, smoking and alcohol consumption. Path analysis was used to determine the role of adiponectin in the association between BW and insulin resistance. Analyses were stratified by BMI (normal, overweight and obese as per WHO criteria).
A significant indirect effect of BW on insulin resistance via adiponectin was found only in men with normal BW (≥3300g) who had normal BMI (18-25kg/m2) in adulthood. In these men, insulin resistance decreased by β = -0.06 (95% CI, -0.11 to -0.02) compared to men born low BW. Results are adjusted for current BMI, smoking, physical activity, alcohol consumption and socioeconomic status. We did not observe similar effects in men born normal BW but overweight or obese in adulthood.
Adiponectin mediates the inverse association between BW and insulin resistance only in men of normal BMI. The mediating effect of adiponectin on insulin resistance is absent in overweight and obese men. This study explains possible mechanisms that influence the inverse relationship of BW and insulin resistance.

PII-229

Birth Weight Is Reduced by Late Gestation Protein Restriction in Sheep. Ali Kiani, Saied Mohamadzade, Masomeh Ghaedrahmati. *Animal Science Group, Lorestan University, Islamic Republic of Iran.*
Birth weight is one of the important factors in predicting animal health and performance which is affected by different stimuli including late gestation nutrition. However, among different nutrients (energy, protein and minerals), a large body of research has been done about effects of level of intake or energy restriction during late gestation on birth weight ([1-3]) and less attention has been given to late gestational protein restriction..
Therefore, we studied the effects of late gestational protein restriction on birth weight and morphological characteristics (size of head, body length and height) of lambs.
Forty single pregnant ewes were fed with *iso*-energitic diet (4.6 Mcal digestible energy) but different in amount of crude protein; either 174 g protein (CONTROL) or 116 g protein (RESTRICTED) during the last four weeks *pre-partum*. At parturition, lambs birth weight and morphological characteristics were measured.
Lambs born in RESTRICTED group (4.9±0.10 kg) were significantly ($p<0.05$) lighter than that in CONTROL (4.4±0.13). No significant difference was found between two groups in respect to morphological characteristics. In addition, there was no relationship between morphological characteristics and lambs gender.
In conclusion, result showed that late gestational protein restriction decreases lamb's birth weight in sheep.

PII-230

Prenatal Growth and Metabolic Syndrome Components in Children. Francisco Mardones, Luis Villarroel, Salesa Barja, Pilar Arnaiz, Oscar Castillo, Marcelo Farias, Angelica Dominguez. *Public Health, Pediatrics, Nutrition and Obstetrics, Pontificia Universidad Catolica de Chile, Santiago, Chile.*
To study the association of prenatal growth with Metabolic Syndrome (MS) components and insulin resistance (IR) in children of a middle income country.
A cohort study was designed linking information on MS components and IR of 10-15 years old children with registers at birth of weight (BW), length (BL), and gestational age (GA). During 2009-2010, 2,174 children were recruited at school in Santiago city. Perinatal data were obtained after standardized measurements performed by trained personnel at the maternity hospital. Examinations included anthropometry and blood pressure, as well as auto-report of pubertal status. While fasting, a blood sample was taken

to determine lipids (enzymatic colorimetric tests), glycemia (hexoquinase), insulin (quimioluminiscence) and HOMA. Pearson correlation, chi-squared test and stepwise multiple regression were used.
2,152 children had complete information at birth. Pearson correlations were significant or tending to be significant: a) for BW they were: with waist circumference: 0,113 ($p < 0.0001$) and with HOMA: -0.048 (p 0.027); b) for BL they were: with waist circumference: 0.069 (p 0.001) and with HOMA: -0.059 (p 0.006); c) for GA they were: with number of risk factors from the MS: -0.067 (0.002), with systolic blood pressure: -0.058 (p 0.007), and with HOMA: -0.042 (p 0.053). In the contingency tables: 1) proportions with high waist circumference and high blood pressure were higher for BW <2500 g (p 0.004 and p 0.020, respectively), 2) proportion with high blood pressure was higher for BL <50 cm (p 0.002), 3) proportion with high blood pressure was higher for GA <37 weeks (p 0.020). Stepwise regressions had the following results: a) high waist circumference was inversely associated to GA (p 0.003) and positively associated to BW ($p < 0.0001$), b) systolic hypertension was inversely associated to GA (p 0.007) and c) IR was inversely associated to BL (p 0.006).
Non-optimal prenatal growth seems to inversely predispose to higher risks of high waist circumference, hypertension, and IR in school-age children. BW was positively associated to waist circumference in the stepwise regression but children in the <2500 g BW category presented a higher proportion of high waist circumference than those in the BW 2500 g and over category. This information is reported for the first time in Chile.

PII-231

Inequalities in Height Growth Trajectories in Childhood: 2004 Pelotas Birth Cohort Study. Alicia Matijasevich[1], Laura D. Howe[2,3], Kate Tilling[2], Iná S. Santos[1], Aluísio J.D. Barros[1], Debbie A. Lawlor[2,3]. *[1]Post-graduate Programme in Epidemiology, Federal University of Pelotas (UFPel), Rio Grande do Sul, Brazil; [2]School of Social and Community Medicine, University of Bristol, United Kingdom; [3]MRC Centre for Causal Analyses in Translational Epidemiology, University of Bristol, United Kingdom.*
Socioeconomic inequalities in attained height have been reported in many countries. The aim of this study was to explore the age at which socioeconomic inequalities in child height emerge among children from a middle-income country.
Using data from the 2004 Pelotas Cohort study from Brazil we modelled individual height growth trajectories in 2106 boys and 1947 girls from birth to 48 months using a linear spline mixed effects model. Differences in height trajectories by maternal education were investigated. We examined the associations of maternal education on birth length and lenght/height growth and explored the effect of adjusting for a number of potential confounders and mediators factors on these associations.
We showed linear and positive associations of maternal education with birth length and length/height growth rates in the first four years of life (lower birth length and rates of childhood growth in children whose mothers had lower educational levels). By age four years the mean height of boys was 101.06cm (SE=0.28) in the lowest and 104.20cm (SE=0.15) in the highest education category. Among girls the mean height was 100.02cm (SE=0.27) and 103.03cm (SE=0.15) in the lowest and highest education categories, respectively. For both boys and girls there was on average a 3cm difference between the extreme education categories. Adjusting for maternal height reduced the observed birth length differences across maternal education categories but differences in postnatal growth rates persisted. Other factors explained little of the observed associations.
Our data demonstrate an increase in the absolute and relative inequality in height after birth indicating that height inequality, which was already present at birth, widened considerably through childhood growth. These findings differ from previous studies in high income countries where inequalities in height at birth persist, but do not widen, postnatally. Hence our results highlight the importance of postnatal environment on infant and childhood growth in a middle income setting.

PII-232

Early Diet Quality in a Longitudinal Study of Australian Children: Associations with Nutrition and Body Mass Index Later in Childhood and Adolescence. Claire E. Meyerkort[1], Wendy H. Oddy[2], Therese A. O'Sullivan[2], Jennifer Henderson[1], Craig E. Pennell[1]. *[1]School of Women's and Infants' Health, The University of Western Australia, Perth, Australia; [2]Telethon Institute of Child Health Research, The University of Western Australia, Perth, Australia.*
Obesity has origins extending to antenatal and early postnatal periods; however, the relationship between early postnatal diet and subsequent obesity is not well defined.
The aims of this study were to determine whether early childhood dietary quality was associated with (a) infant and adolescent nutrition and (b) Body Mass Index (BMI) in childhood and adolescence. The degree to which early nutrition and growth factors determine BMI throughout childhood and adolescence was also explored.
This research was conducted using the Raine Study, a longitudinal survey of Australian children, which involved data collection from the 16th week of gestation to 17 years of age. A dietary quality index, the Raine Eating Assessment in Toddler (EAT) Score, was assigned to 2562 cohort members to assess early nutrition. Linear regression analyses determined that breastfeeding was associated with dietary quality at one to three years.
Dietary elements at 14 years of age were related to earlier dietary quality. There were no consistent associations between early dietary quality and BMI at three, five, eight, ten, fourteen or seventeen years. In contrast, birth weight and infant weight gain were significantly associated with BMI at these ages.
Although early dietary patterns are associated with aspects of diet in adolescence, this study suggests that birth weight and early growth are more important determinants of BMI later in life, than early diet and nutrition. While optimizing early diet has potential to influence later nutrition, interventions focussing on early weight gain may have a greater impact on the obesity epidemic.

PII-233

Body Fat in Young Singaporean Infants: Development of Body Fat Prediction Equations in Asian Newborns. Izzuddin B. Mohd Aris[1], Mya T. Tint[1,4], Shu E. Soh[1,4], Jenny Liu[1], Amutha Chinnadurai[1,4], Seang Mei Saw[1], Kenneth Kwek[2], Yap Seng Chong[1,4], Peter D. Gluckman[3], Keith M. Godfrey[5], Fabian Yap[2], Yung Seng Lee[1,3,4]. *[1]National University of Singapore, Singapore; [2]Kandang Kerbau Hospital, Singapore; [3]Singapore Institute of Clinical Sciences, Singapore; [4]National University Health System, Singapore; [5]University of Southampton, United Kingdom.*
Develop body fat prediction equations in Asian neonates and devising a strategy to determine the %BF in an Asian birth cohort.
%BF were measured by air displacement plethysmography (PEA POD®; Life Measurement Inc, Concord, CA) with triceps and subscapular skinfold (SFT) measurements in 171 infants from the birth cohort study "Growing Up in Singapore Towards healthy Outcomes" (GUSTO) at visit 2 (day 5-22 of life). Using data from 87 neonates (50.6% males), prediction equations were derived using forward stepwise regression model with PEAPOD-derived %BF as reference standard. The prediction equations were validated in another 84 newborns (validation group) to obtain SFT-derived %BF, and compared to PEAPOD-derived %BF. To assess the agreement of the two methods, Pearson correlation, linear regression and Bland-Altman analyses were carried out using SPSS(version 17.0).
The best fit linear regression model of %BF for male infants had SFT as a significant predictor (%BF = -0.614 + 0.973*ΣSFT);for female infants, the significant predictors were body mass and SFT (%BF = -8.368 + 0.584*ΣSFT + 4.158*body mass). %BF predicted by new SFT equations was significantly correlated to PEAPOD-derived %BF on infants from the validation group ($r = 0.584$, $p < 0.0005$). By Bland-Altman analysis, the 95% limits of agreement is -7.42%, 7.12% BF. Significant negative trend in %BF differences were noted as %BF varied ($r = -0.496$, $p < 0.0005$; $\beta = -0.55$, $p < 0.0005$). The mean difference between SFT-derived %BF using our equation and PEAPOD derived %BF is only -0.15% ($p = 0.713$),significantly lesser than SFT-derived %BF using a published prediction equation (Slaughter *et al.*) with PEA POD derived %BF, which showed mean difference of 1.05% ($p < 0.0005$).

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This newly-derived %BF prediction equation is in agreement with PEAPOD-derived %BF, though the new SFT equations tend to underestimate %BF for infants with higher body fat and overestimate %BF for infants with low body fat. We can use this prediction equation to estimate the %BF in the 1000 other newborns in the GUSTO cohort who only have skinfold and not PEA POD measurement.

PII-234

Body Fat Estimated from Skinfold Thickness in Asian Neonates. Izzuddin B. Mohd Aris[1], Mya T. Tint[1,4], Shu E. Soh[1,4], Jenny Liu[1], Amutha Chinnadurai[1,4], Seang Mei Saw[1], Kenneth Kwek[2], Yap Seng Chong[1,4], Peter D. Gluckman[3], Keith M. Godfrey[5], Fabian Yap[2], Yung Seng Lee[1,3,4]. *[1]National University of Singapore, Singapore; [2]Kandang Kerbau Hospital, Singapore; [3]Singapore Institute of Clinical Sciences, Singapore; [4]National University Health System, Singapore; [5]University of Southampton, United Kingdom.*

Determine the accuracy of percentage body fat (%BF) derived from skinfold thickness (SFT) measurements in neonates.

Preliminary analyses of data collected from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort study were done. %BF of 171 infants at visit 1 (day one of life) and visit 2 (day 5-22 of life; 10.28 ± 2.72 days) was derived from air displacement plethysmography (PEA POD®) as a reference standard; and triceps and subscapular skinfold measurements were obtained. We compared %BF derived from established skinfold prediction equations (Slaughter *et al.* 1988) with %BF measured by PEA POD® using linear regression and Bland-Altman analyses (SPSS, version 17.0).

Results demonstrate significant SFT and PEA POD derived %BF correlation at visit 1 ($r = 0.523$, $p<0.0005$) and visit 2 ($r = 0.587$, $p<0.0005$). However, significant within-day %BF differences between the two methods were noted on visit 1 (0.85 ± 3.21%, $p<0.0005$) and visit 2 (1.05 ± 3.50%, $p<0.0005$). By Bland-Altman analysis, the 95% limits of agreement were -5.44%,7.13% for visit 1, and -5.81%,7.92% for visit 2. Significant negative trend in %BF differences as %BF varied were noted between SFT estimates and PEA POD for visit 1 ($r=-0.43$, $p<0.0005$; $\beta = -0.51$, $p < 0.0005$) and visit 2 ($r= -0.33$, $p<0.0005$; $\beta = -0.45$, $p < 0.0005$).

As a measurement of %BF, SFT is best measured at one week rather than on the first day of life. The Bland-Altman diagrams show that SFT tend to underestimate %BF for infants with higher %BF, especially for measurements taken on the first day of life in contrast to measurements from 5-22 days. The Bland Altman analyses showed possible differences of up to 7.9% between the two measures, with SFT measures tending to overestimate at lower %BF, and underestimate at higher %BF.

PII-235

Leptin Plasma Levels at Birth and Postnatal Growth Pattern Are Involved in Visceral Adipose Tissue Accumulation in Adulthood. Norma B. Ojeda[1], John H. Dasinger[1], Lee F. Tull[2], Haiyan Zhang[2], Laura Bufkin[3], James Martin[3], Barbara T. Alexander[2], Phil G. Rhodes[1]. *[1]Pediatrics Department, University of Mississippi Medical Center, MS, USA; [2]Physiology Department, University of Mississippi Medical Center, MS, USA; [3]Obstetrics and Gynecology Department, University of Mississippi Medical Center, MS, USA.*

Several studies suggest that postnatal growth pattern in low birth weight (LBW) babies may be is involved in visceral adipose tissue (VAT) accumulation in adulthood, yet the exact mechanism is not clear. The objective of this translational study was to determine whether plasma levels of leptin, a hormone with neuroendocrine actions, regulated postnatal growth pattern in LBW individuals and affects visceral adipose tissue accumulation in adulthood.

The clinical protocol used a prospective observational cohort study in newborn babies; the experimental protocol used a rodent model of LBW induced by placental insufficiency.

Human LBW babies exhibited an accelerated postnatal growth when compared with normal birth weight (NBW) babies within three weeks of postnatal life. (15±8 vs. -56±30 g; $P<0.05$, LBW vs. NBW; respectively). Moreover, a negative correlation for plasma leptin levels at birth with post-natal weight gain was observed in LBW babies (Spearman r= -0.6734). Rodents LBW offspring also demonstrated accelerated postnatal growth compared to NBW offspring within eight weeks of postnatal life (6±0.8 vs. 4±0.9 g; $P<0.05$, LBW vs. NBW; respectively). Furthermore, a negative correlation was observed for plasma leptin levels at birth with postnatal growth in LBW rodents (Pearson r= -0.9454). In the experimental model, LBW rodents demonstrated an increased VAT at six months of age relative to NBW (23±3 vs. 36±5 g/K; $P<0.05$, NBW vs. LBW; respectively).

Thus, the parallelisms between human and animal studies suggest that plasma leptin levels at birth are associated with accelerated postnatal growth which may contribute to increased accumulation of visceral adipose tissue in adult LBW offspring.

PII-236

A Postnatal Diet with a More Breast Milk-Like Lipid Matrix Markedly Reduces Body Fat Accumulation in Adult Mice. Annemarie Oosting[1], Eefje Engels[1], Diane Kegler[1], Marieke Abrahamse[1], Eline M. van der Beek[2]. *[1]Danone Research - Centre for Specialised Nutrition, Wageningen, Netherlands; [2]Danone Research, Singapore.*

Dietary fat quality during postnatal life plays an important role in the development of body composition later in life. We previously reported that fatty acid composition (i.e. n3 LCP enrichment[1] as well as n6 PUFA reduction[2] prevents excessive fat accumulation in adult mice). The current study investigates another feature of dietary fat quality, namely the physical properties of lipid globules. We developed an infant milk formula with a complex lipid matrix (Nuturis®) consisting of large lipid globules coated by a phospholipid layer, more closely resembling the lipid droplets in breast milk, and investigated long term effects on fat accumulation in adult mice.

Male C57Bl/6j mice were fed with a diet containing either Nuturis® or standard infant formula (CTR) from postnatal day (PN)16 to 42. Subsequently, the mice were challenged with a moderate Western style diet (WSD) during adolescence and adulthood until dissection at PN98. A reference group was included with mice raised on CTR switching to standard rodent chow instead of WSD from PN42 onwards. Body composition was monitored by dual x-ray absorptiometry at PN42, 70, and 98. Epididymal depots were analyzed for adipocyte number and size distribution.

At PN98, total body weight was lower for Nuturis®group compared to the CTR group. This difference was entirely due to less fat accumulation, since lean body mass was similar in both groups. In accordance, visceral and subcutaneous fat depots were smaller in mice raised on Nuturis®group compared to CTR diet. Moreover, total body weight and fat mass of the Nuturis®group, but not CTR group, was similar to the reference group, which was not challenged by WSD. In contrast to CTR and again more in line with the reference group, the Nuturis® group had a larger proportion of small adipocytes. Adipocyte number, however, was similar in all groups.

Exposure to a more breast milk-like lipid matrix (Nuturis®) early in life prevents excessive fat accumulation when challenged with a moderate WSD during adulthood. The reduced body fat mass accumulation and fat depot weight was due to smaller adipocytes rather than less adipocytes. Future studies should elucidate the mechanism by which Nuturis® prevents excess fat storage in adulthood.

1) Oosting *et al.*, 2010, Pediatric Research 68(6): 494-499
2) Oosting *et al.*, 2011, DOHaD, Portland, USA.

PII-237

Separating Height and Weight in Infancy and Childhood as Predictors of Adult Glucose Tolerance and Body Composition. Clive Osmond[1], Harshpal Singh Sachdev[2], Nikhil Tandon[3], Santosh K. Bhargava[4], Caroline H.D. Fall[1]. *[1]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom; [2]Sitaram Bhartia Institute of Science and Research, New Delhi, India; [3]All India Institute of Medical Sciences, New Delhi, India; [4]Sunder Lal Jain Hospital, New Delhi, India.*

Is it wise to promote the growth of infants and children in low and middle income countries? Whilst infant growth is associated with beneficial outcomes, including increased survival, cognitive development, educational attainment, economic productivity and next-generation birthweight, it may also lead to overweight and consequent adult disease.

1341 men and women aged 30y from a longitudinal study that started in New Delhi, India, in 1969. They had a standard 75g glucose tolerance

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test. Their heights, weights and skin-fold thicknesses were measured and Indian equations were used to estimate their fat and fat-free mass. Women reported age at menarche.

We describe a statistical approach to distinguish linear (skeletal) growth from weight gain (mainly soft tissue growth) in early infancy (birth to 6m), late infancy (6m-2y) and childhood (2-11y), and use it to predict adult glucose tolerance and body composition. "Conditional height gain" is height gain in an interval beyond that which would have been predicted from all measurements of height and weight known at the beginning of the interval. "Conditional weight gain" in defined similarly, but also includes height at the end of the interval in the prediction.

Plasma glucose concentration fell with increasing conditional birth weight and conditional weight gain in late infancy. Birth length, conditional birth weight and conditional gain in both height and weight in all time intervals predicted both adult fat mass and non-fat mass. The height regression coefficients were similar in each age interval, but the weight coefficients tended to increase with age. At birth and in infancy weight coefficients were larger for non-fat than fat mass, whilst the opposite was true for childhood weight coefficients. Birth length, conditional height gain in each interval and conditional weights at birth and in early infancy predicted adult height. Conditional weight gain in childhood was negatively associated with adult height and age at menarche.

Weight gain in infancy, over and above linear growth, was associated with lower 2-h glucose concentration and made a greater contribution to adult non-fat mass than fat mass.

PII-238

Sex-Specific Regulation of Glucocorticoid Exposure in Human Preterm Pregnancies by P-Glycoprotein and 11beta Hydroxysteroid Dehydrogenase 2. Nicolette A. Hodyl, Vicki L. Clifton, Michael J. Stark. *Robinson Institute, University of Adelaide, South Australia, Australia.*

Fetal glucocorticoid exposure is tightly regulated across gestation by the placental glucocorticoid barrier. This is comprised in part by the cortisol inactivating enzyme 11beta hydroxysteroid dehydrogenase (11βHSD2) together with multidrug resistant transmembrane efflux proteins, such as P-glycoprotein (P-gp), located on the syncytiotrophoblast. Observations of a greater prophylactic effect of the synthetic glucocorticoid betamethasone in female preterm births compared to males have been demonstrated. We questioned whether this was due to sex-specific alterations in placental P-gp. The aim of this study was to assess placental P-gp together with 11βHSD2 activity in placenta from preterm pregnancies to understand glucocorticoid regulation.

Placental samples were collected from women who delivered preterm (24-36 weeks n=42) or at term (n=11). P-gp mRNA was measured by qRT-PCR and 11βHSD2 activity by radiometric conversion assay. Betamethasone exposure was classified as delivery <72 or >72 hours after maternal steroid administration.

Placental P-gp expression increased exponentially with advancing gestation in the preterm infants (R^2=.364, p=0.018), but expression returned to low levels at term. P-gp expression was inversely correlated with 11βHSD2 activity in females (r=-.664, p=0.018) but not in males. P-gp was not affected by betamethasone exposure.

Antenatal betamethasone does not alter expression of P-gp in preterm placenta. However, P-gp appears to play a strong role in maintaining fetal glucocorticoid homeostasis across gestation. This is particularly evident with a female preterm infant, where our results suggest increased glucocorticoid efflux from the placenta by P-gp when 11βHSD2 activity is at its lowest. This study supports previous observations of greater placental sensitivity and adaptation in the female preterm neonate compared to male.

PII-239

Cardiac Ultrasound in Adults Born at Birth Weight Less Than 1500 Grams. Petteri Hovi[1,2], Maila Turanlahti[1], Sonja Strang-Karlsson[1,2], Karoliina Wehkalampi[1,2], Anna-Liisa Jarvenpaa[1], Johan G. Eriksson[2,3,4,5,6], Eero Kajantie[1,2], Sture Andersson[1]. *[1]Children's Hospital, University of Helsinki, Finland; [2]National Institute for Health and Welfare, Finland; [3]Department of General Practice and Primary Health Care, Institute of Clinical Medicine, University of Helsinki, Finland; [4]Vasa Central Hospital, Finland; [5]Unit of General Practice, Helsinki University Central Hospital, Finland; [6]Folkhalsan Research Centre, Finland.*

Adults born at a very low birth weight (VLBW, <1500 g), in comparison to term born adults, have higher blood pressure and a higher incidence of hypertension. In search for signs of relative hypertrophy in the heart among adults born at VLBW, we measured thickness of the interventricular septum.

As a part of the Helsinki Study of Very Low Birth Weight Adults, we performed cardiac ultrasound (5 MHz-probe) in 92 VLBW subjects (40 men) and 66 term born subjects (29 men) at age 18 to 27 years. We weighed the participants and measured their height and blood pressure. We measured fat free mass by dual-energy X-ray absorptiometry and obtained parental education data by a questionnaire.

As compared to those born at term, the VLBW adults were lighter and shorter and their body surface area was lower: 1.80 (0.20) vs. 1.97 (0.15) m² among men and 1.60 (0.20) vs. 1.70 (0.17) m² among women. The VLBW adults had higher systolic pressure: 125.4 (SD 13.0) vs. 122.9 (10.7) mmHg among men and 116.9 (10.4) vs. 110.3 (7.5) mmHg among women. An age and sex adjusted difference estimate between the VLBW and term-born subjects was 4.8 (95% CI, 1.4 to 8.2) mmHg. Diastolic pressures showed similar results.

Thickness of the left ventricle posterior wall was similar in the groups, whereas the interventricular septum among the VLBW subjects was thinner: 10.6 (SD 1.8) vs. 11.7 (1.7) mm among men and 9.4 (1.6) vs. 10.0 (1.1) mm among women. The age and sex adjusted difference was 0.8 (95% CI, 0.3 to 1.3) mm. The differences persisted after further adjustments for body surface area, BMI, fat free mass, or parental education.

Interventricular septum in adults born at VLBW is thinner than in peers born at term. Thus, cardiac hypertrophy does not seem to accompany the observed higher blood pressure in VLBW subjects in young adulthood.

PII-240

Preterm Birth and Insulin Sensitivity in Young Adult Life: The Helsinki Study of Very Low Birth Weight Adults. Eero Kajantie[1,2], Sonja Strang-Karlsson[1,2], Petteri Hovi[1,2], Karoliina Wehkalampi[1,2], Jari Lahti[3], Katri Räikkönen[3], Anna-Liisa Järvenpää[2], Johan G. Eriksson[1,4], Sture Andersson[2]. *[1]Diabetes Prevention Unit, National Institute for Health and Welfare, Helsinki, Finland; [2]Children's Hospital, Helsinki University Central Hospital, Helsinki, Finland; [3]Institute of Behavioural Sciences, University of Helsinki, Helsinki, Finland; [4]Department of General Practice and Primary Health Care, University of Helsinki, Helsinki, Finland.*

Preterm birth is associated with impaired glucose regulation and increased risk of type 2 diabetes in adult life. The mechanisms of this association are poorly known. We studied insulin sensitivity and secretion in adults born at very low birth weight (VLBW; <1500 g).

The subjects were 104 adults born at VLBW (mean gestational age 29.3 weeks; range 24.0 to 35.6, birth weight 1127 g; 600 to 1480) and 100 controls born at term not small-for-gestational age (SGA), from a group matched for sex, age and birth hospital. Their mean age was 25.0 years. We performed a 14-sample intravenous glucose tolerance test and calculated with insulin sensitivity (SI), first-phase insulin release (AIR) and their product, disposition index, by Minimal Model (Minmod Millennium®).

Compared with controls, VLBW adults had lower insulin sensitivity (mean difference -11.7%, 95% CI -22.0 to 0.0%, adjusted for sex, age and body mass index) and higher acute insulin release (18.7%; 3.3 to 36.3%). The association with insulin sensitivity attenuated to non-significance when further adjusted for parental education. Disposition index was similar between the groups. There was no difference between the 39 VLBW adults born SGA and the remaining 65 VLBW adults born appropriate for gestational age.

Young adults born preterm at VLBW are more insulin resistant than their term-born peers with a similar body mass index. This is in part compensated by their higher insulin secretion.

PII-241

Preterm Birth at Very Low Birth Weight and Nutrient Intake in Adult Life. Nina Kaseva[1,2], Karoliina Wehkalmpi[1,2], Katri Hemiö[1], Petteri Hovi[1,2], Anna-Liisa Järvenpää[2], Sture Andersson[2], Johan G. Eriksson[1,2,3], Jaana Lindström[1], Eero Kajantie[1]. *[1]National Institute for Health and Welfare, Finland; [2]Helsinki University Central Hospital, Finland; [3]University of Helsinki, Finland.*

The period after severely preterm birth is characterized by immaturity-associated illness and inadequate nutrition and thus constitutes a model of early nutritional deprivation. We studied dietary intake in healthy young adults born preterm at very low birth weight (VLBW, <1500g).

151 unimpaired young adults, aged 19-27 years, born at VLBW and 156 age-, sex-, and birth hospital-matched term-born controls completed a 3-day food record and used a picture booklet with food portions to estimate portion sizes. A nutritionist interviewed the participants after completion of the food record. Nutrient intakes were calculated with a dietary analysis program based on the national FINELI® database. A subset of participants underwent dual-energy x-ray absorptiometry for body composition measurement. Data were analyzed by multiple linear regression.

As compared with controls, the VLBW subjects reported 194.1 kcal lower daily energy intake (95% CI; -311.9 to -76.4 adjusted for age, sex, socio-economic status, daily smoking and perinatal factors), which was due to their smaller body size; (mean difference adjusted in addition for height and BMI -82.6 kcal/d, 95% CI; -206.7 to 41.5). There was no difference between groups in the proportion of energy from carbohydrates [-1.0% (95% CI; -2.8 to 0.9), fat [1.2% (95% CI;-0.5 to 3.0)] or protein [-0.6% (95% CI; -1.5 to 0.4)]. The mean values for the proportions of daily energy intake in VLBW and controls from carbohydrate were as follows: 46.3% (SD 7.4%) and 47.2% (8.4%), for protein 16.1% (3.8%) and 16.2% (3.9%) and for fat 34.7% (7.0%) and 33.4% (7.1%). Compared with controls, VLBW participants had lower intake of calcium -219.8 mg (95% CI; -328.7 to -110.9) and cholesterol -29.9 mg (95% CI; -49.3 to -10.6). Vitamin D intake was 3.7 µg/d (SD 2.6) in VLBW and 4.4 µg/d (3.6) in control subjects [mean difference -0.8 (95% CI -1.6 to -0.03)].

We found no evidence for altered macronutrient intake in young adults born preterm at VLBW. Further analysis of micronutrient intake is ongoing. Calcium intake was lower in the VLBW group; this may be of importance as increased risk for osteoporosis has been linked to preterm birth at VLBW.

PII-242

Young Adults Born Preterm at Very Low Birth Weight Undertake Less Physical Activity Than Their Peers Born at Term. Nina Kaseva[1,2], Karoliina Wehkalampi[1,2], Sonja Strang-Karlsson[1,2], Minna Salonen[1], Anu-Katriina Pesonen[3], Katri Räikkönen[3], Tuija Tammelin[4], Petteri Hovi[1,2], Jari Lahti[3], Kati Heinonen[3], Anna-Liisa Järvenpää[2], Sture Andersson[2], Johan G. Eriksson[1,2,3,5], Eero Kajantie[1,2]. *[1]National Institute for Health and Welfare, Finland; [2]Helsinki University Central Hospital, Finland; [3]University of Helsinki, Finland; [4]LIKES Research Center for Sport and Health Sciences, Finland; [5]Folkhälsan Research Centre, Finland.*

A recent meta-analysis (Andersen *et al.* PLoS One 2009;4:e8192) showed that the association between birth weight and undertaking leisure-time physical activity (LTPA) is very weak within the normal birth weight range, but both low and high birth weights may be associated with low rates of self-reported LTPA. We assessed LTPA and its components in healthy young adults born preterm at very low birth weight (VLBW, <1500g) compared with term-born controls.

We studied 94 unimpaired young adults, aged 21-29 years, born at VLBW and 101 age-, sex-, and birth hospital-matched term-born controls. The participants completed a validated 30-item 12-month physical activity questionnaire and the NEO-Personality Inventory measuring the "big five" personality traits. Yearly frequency, total time, total volume and energy expenditure of conditioning and non-conditioning LTPA and commuting physical activity were compared between VLBW and term-born subjects. A subset of participants underwent dual-energy x-ray absorptiometry for body composition measurement. Data were analyzed by multiple linear regression.

Compared with controls, VLBW participants had lower frequency [-38.5% (95% CI; -58.9, -7.7)], total time [-47.4% (95% CI; -71.2, -4.1)], total volume [-44.3% (95% CI; -65.8, -9.2)] and energy expenditure [-55.9% (95% CI; -78.6, -9.4)] of conditioning LTPA when adjusted for age, sex, body mass index, smoking, parental education and personality traits. Adjusting for lean body mass instead of body mass index attenuated the difference. There were no differences in non-conditioning LTPA or commuting physical activity.

Compared with term-born controls, unimpaired VLBW adults undertake less frequent LTPA with lower total time and volume of exercise resulting in lower energy expenditure. This finding reinforces previous suggestions that physical activity is programmed early in life. It also underlies the importance of early promotion of physical activity in the prevention of chronic non-communicable diseases in VLBW individuals.

PII-243

Does Preterm Birth Affect Vascular Health Status in Young Adulthood? Gerthe F. Kerkhof, Petra E. Breukhoven, Ralph W.J. Leunissen, Ruben H. Willemsen, Anita C.S. Hokken-Koelega. *Pediatrics, subdivision of Endocrinology, ErasmusMC/Sophia Children's Hospital, Netherlands.*

Both preterm birth and small birth size for gestational age (SGA) have been associated with an increased risk for developing cardiovascular diseases (CVD), but controversies still exist. We investigated the effect of preterm birth on several parameters of vascular health status. We hypothesized that preterm birth is associated with increased risk for CVD in young adulthood, independent of size at birth.

In 406 young adults of the PROGRAM/PREMS study, aged 18-24 yr, the effect of preterm birth (gestational age <36 weeks) on systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure, blood pressure variability, heart rate, Pulse Wave Velocity (PWV), and carotid Intima Media Thickness (cIMT) was analyzed. To study the differential effect of preterm and SGA birth on vascular health, these parameters were also analyzed in subgroups: young adults born small for gestational age with short stature (SGA-S) or normal stature (SGA-CU), born either preterm or term, and young adults born appropriate for gestational age with normal stature (AGA), born either preterm or term.

Subjects born preterm had a higher SBP, pulse pressure, blood pressure variability, heart rate, and a lower DBP than subjects born at term. In the total group, the continuous variable gestational age was inversely associated with SBP via an increased heart rate, inversely associated with pulse pressure and blood pressure variability, and positively associated with DBP, also after adjustment for confounders. There was no effect of gestational age on PWV and cIMT. Of all vascular health parameters, higher pulse pressure affected cIMT the most. Subgroup analyses showed that all preterm subgroups had a significantly higher pulse pressure and DBP variability than the reference group (AGA subjects born at term), but lower DBP. SGA-CU and AGA subjects born preterm had a higher heart rate than the reference group. There were no differences in vascular health parameters between the SGA and AGA groups born at term.

Our results show that young adults born preterm have a less favorable vascular health status than those born at term, independent of birth size. Subgroup comparisons showed that the effect of preterm birth on vascular health is likely not to be due to SGA birth or catch up growth.

PII-244

Effects of Maternal Protein Restriction and Short Term Statin Treatment on the Vascular Function of the Offspring. P. H.M. Keskivali[1], N. P. Curzen[1,2], G. F. Clough[1], C. Torrens[1]. *[1]Faculty of Medicine, University of Southampton, United Kingdom; [2]Wessex Cardiothoracic Unit, University of Southampton, United Kingdom.*

In rats maternal protein restriction during pregnancy leads to cardiovascular dysfunction in the offspring. Administration of statins (HMG Co-A reductase inhibitors) from weaning onwards can improve this (Torrens *et al.*, 2009). The aim of this study was to determine if early age short term statin treatment improves cardiovascular function in offspring of protein restricted dams.

Wistar rat dams were fed isocaloric control (C, 18% casein) or protein restricted (PR, 9% casein) diet during pregnancy and standard chow during

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suckling. At weaning (3 weeks) offspring either received atorvastatin in drinking water (10mg/kg per day) or acted as control giving four offspring groups; control (C), control+statin (CS), protein restricted (PR) and PR+statin (PRS). At five weeks of age offspring blood pressure (BP) was recorded by tail cuff plethysmography and aorta reactivity was assessed post mortem using wire myography. Cumulative concentration response curves were constructed to phenyleprine (PE), acetylcholine (ACh) and sodium nitroprusside (SNP). Results are presented as mean±SEM. Differences were assessed by one-way ANOVA, significance assumed at p£0.05.

No differences were seen in BP across the groups. Statin treatment reduced the maximal constrictor response to PE in CS and PRS as compared to controls in male (g: C 2.27±0.09, n=5; CS 1.65±0.15, n=5; PR 1.86±0.07, n=4; PRS 1.58±0.15, n=4, p<0.01) but not female offspring. No differences were seen in maximal relaxation response to ACh in male (% of constriction: C 75.2±6.4, n=5; CS; 59.1±5.3, n=5; PR 70.8±9.5, n=4; PRS 82.4±8.3, n=4) or female (% of constriction; C 76.4±5.9, n=4; CS 71.4±5.3, n=5; PR 76.2±6.4, n=4; PRS 83.9±4.9, n=4) offspring at five weeks. Smooth muscle function as assessed by relaxation to SNP was similar in all groups.

These results show that short term statin treatment reduces maximal constriction to PE regardless of pre-natal diet. At five weeks endothelial function was unaffected by either maternal diet or short term statin treatment, suggesting a later onset of endothelial dysfunction and beneficial effects of statins.

Torrens *et al.*. (2009) *Hypertension* 2009; **53**:661-667.

PII-245

Ouabain Decreases the Risk of Rat Hypertension and Renal Disease by Rescuing Kidney Development during Intrauterin Growth Restriction.

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Low birth weight due to intrauterine growth restriction (IUGR) is associated with an increased risk of hypertension and end stage renal disease in later life. Overwhelming evidence suggests that this is because of IUGR endangers kidney development and results in an irreversible loss of nephrons. Yet, no drug available that would alleviate the effects of IUGR on nephron formation. In our previous work in vitro study, using explanted rat embryonic kidneys, we found that ouabain, the Na,K-ATPase ligand, by triggering a calcium–nuclear factor- κB signal, protected embryonic kidney development from IUGR. Here we report that ouabain rescues embryonic kidney development in vivo study and decreases the risk of hypertension and end stage renal disease in later life.

Protocol The Animal Welfare Board at Nanjing University approved the protocol for this investigation. Pregnant SD rats were given either a low (9%) or a normal (18%) protein diet immediately after mating. Under Isofluran anaesthesia, osmotic pumps (Alzet Osmotic Pumps), delivering either ouabain dissolved in PBS (15 μ g ouabain per kg body weight per day) or vehicle, were implanted subcutaneously on the second day of pregnancy. **Glomeruli counting** Half of the embryos were killed at birth and their kidneys were processed for stereological examination of glomerular number. Stereological analysis was performed using a modified form of the disector method. **Blood Pressure (BP)** Half of the embryos were followed 18 monthes, SBP was measured each month by tail plethysmography (Softron,Japan). **Renal function** Serum urea nitrogen and creatinine were measured using the VetACE clinical chemistry system(Hitachi 7600, Japan).

In ouabain treated rats, the embryos' nephrons were increased compare to vehicle treated rats. We followed up the left embryos for 18 months, measuring the blood pressure, serum urea nitrogen and serum creatinine. We found that in ouabain treated group, the risk of hypertension and end stage renal disease is decreased compare to vehicle treated group.

Thus we have identified a novel medicine by which kidney development can be protected under IUGR.

PII-246

Effects of a Direct Fetal Amino Acid Infusion on Oxygenation and Acid-Base Balance in Sheep.

Anne M. Maliszewski, Monika Gadhia, Meghan O'Meara, Stephanie Thorn, William Hay, Paul J. Rozance, Laura D. Brown. *Pediatrics, University of Colorado, CO, USA.*

Maternal amino acid infusion in pregnant sheep results in competitive inhibition of amino acid transport across the placenta, increased fetal oxygen (O_2) consumption, fetal hypoxia and acidosis. A direct fetal amino acid infusion, bypassing competitive inhibition of amino acid transport across the placenta, will increase fetal O_2 consumption but preserve acid-base balance.

Late gestation singleton fetal sheep were intravenously infused with a complete amino acid mixture (AA, n=8) or saline (C, n=10) for an average of 12 days. The effects of treatment and day of infusion on fetal arterial plasma branched chain amino acid (BCAA) concentrations; pH; blood gasses; hematocrit; plasma lactate, glucose, and insulin concentrations, and glucose/O_2 quotient. On final day of infusion, umbilical blood flow, fetal O_2 consumption, and net rates of fetal glucose, lactate and amino acid uptakes from the placenta were measured.

Fetal [BCAA] were increased by 50% in AA vs. C (P<0.005). Glucose decreased in AA (22.6±1.5 baseline vs. 18.0±1.2 mg/dl final infusion day, P<0.0005). Fetal pH, pCO_2, hematocrit, hemoglobin-O_2 saturation, and blood O_2 content did not change. Fetal arterial blood pO_2 decreased in AA from baseline (18.9±0.7 mmHg) on days 5 (16.5±1.4 mmHg, P<0.05) and 8 (16.3±1.3 mmHg, P<0.05) then returned to baseline. Fetal arterial plasma lactate concentrations increased in the AA group initially (2.05±0.13 baseline vs 4.41±1.3 mmol/L on day 7, P<.005) then returned to baseline. The fetal glucose/O_2 quotient decreased in AA (P<0.05) but not C.

On final day of infusion, fetal arterial essential amino acid concentrations increased by 32%; concentrations of non-essential amino acids did not change.Net fetal uptake of most amino acids were not inhibited by amino acid infusion. Net fetal glucose uptake was lower in AA vs. C (2.52±0.36 vs 3.86±0.11 mg/kg/min, P<0.05). Umbilical blood flow, net fetal lactate uptake, and O_2 consumption rates did not change in AA.

Prolonged infusion of amino acids directly into fetal sheep increased essential fetal arterial amino acid concentrations but did not inhibit net amino acid uptake. Fetal O_2 consumption was not increased and acid-base balance was preserved. We speculate that decreased net fetal glucose uptake and fetal glucose/O_2 quotient in the AA group is due to increased fetal amino acid oxidation substituting for glucose oxidation.

PII-247

Impaired Insulin Receptor Tyrosine Phosphorylation in Pancreatic Islets from MSG-Obese Mice Is Improved by Early Swim Training.

Rosiane A. Miranda[1], Renato C.S. Branco[1], Luiz F. Barella[1], Ana E. Andreazzi[2], Júlio C. de Oliveira[1], Maria C. Picinato[3], Paulo C.F. Mathias[1]. *[1]Department of Cell Biology and Genetics, State University of Maringá, Paraná, Brazil; [2]Department of Biology, Federal University of Juiz de Fora, Minas Gerais, Brazil; [3]Department of Biologic Sciences, Foundation of Higher Teaching of Passos- State University of Minas Gerais, Minas gerais, Brazil.*

Obesity produced by hypothalamic injury, such as that obtained by neonatal treatment with monosodium L-glutamate (MSG), is characterized by hyperinsulinaemia without normal glucose uptake, which is due to severe tissue insulin resistance from dysfunction of the insulin receptor (IR) and its downstream signalling pathways. Insulin receptor substrate-1 (IRS-1) plays a key role in transmitting signals from the insulin receptor in several tissues including pancreatic beta cells. In both humans and experimental animals, deregulation of glucose homeostasis and insulin sensitivity/secretion were improved by exercise training. Nevertheless, the mechanisms underlying the changes in insulin signalling pathways are currently unknown.

Swim training of 90-day-old MSG-mice was used to evaluate whether signalling pathways of the IR and IRS-1 in islets are involved with the insulin resistance and glucose intolerance observed in this obese animal model.

IR tyrosine phosphorylation (pIR) was reduced by 42% in MSG-obese mice (MSG, 6.7±0.2 arbitrary units (au); control, 11.5±0.4au); on the other hand, exercise training increased pIR by 76% in MSG-mice without affecting control mice (MSG, 11.8±0.3; control, 12.8±0.2au). Although the treatment

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with MSG increased IRS-1 tyrosine phosphorylation (pIRS-1) by 96% (MSG, 17.02±0.6; control, 8.7±0.2au), exercise training also increased it in both groups (control, 13.6±0.1; MSG, 22.2±1.1au).
Obesity has harmful effects on the insulin receptors from pancreatic islets by reducing their tyrosine phosphorylation, and exercise training can modulate this effect.

PII-248

Trends for Displacement of Morbidity and Mortality Curves to More Advanced Age Categories and for Decrease in Female Fraction: Is There a Contribution of Developmental Programming? Viktor I. Goudochnikov. *Membership, International Society for DOHaD, Brazil.*
Earlier we have studied the dynamics of morbidity and mortality caused by diseases of three groups: cardiometabolic, neuropsychiatric and various cancer types, in three brazilian states of southern region during the period 2001-2004 (Goudochnikov, 2009). In present work we compared the morbidity and mortality patterns for two 3-year periods: 1998-2000 and 2005-2007.
Epidemiologic data for cardiometabolic and respiratory diseases were retrieved from brazilian national database DataSus. At first, annual raw data for both sexes together were recalculated for each age decade as a percentage of total morbidity or mortality in all age categories. After that, female fraction was calculated for each age decade in per cent of total morbidity or mortality for both sexes. Finally, arithmetic means and standard errors of epidemiologic parameters were found for both 3-year periods evaluated.
These procedures generated the curves of morbidity and mortality that showed a clear trend for displacement to more advanced age categories, at least for some diseases and to a less degree for mortality, when comparing two rather distant chronological periods. Besides, there was a tendency to decrease in female fraction for some disorders. The question emerges: what are the reasons of such displacement and decrease and is there any contribution of developmental programming/imprinting phenomena? Here we discuss the limitations of studying epidemiologic aspects of DOHaD problematics in a long-term perspective, especially in highly heterogeneous human populations, changing over prolonged chronological periods.
On the other hand, the relative stability of morbidity and mortality patterns allows us to suggest that dynamics of epidemiologic parameters may be used for conclusions like that was made by us earlier on the absence of evidence for unique general scheme of aging. Obviously enough, our data should be extended in near future to other chronological periods and different pathologies, as well as for other brazilian states and regions.

PII-249

Withdrawn by Author

PII-250

Parenting Control Practices over Children's Eating Habits May Be a Focus for Public Health Interventions. Megan Jarman[1,2], Georgia Ntani[2], Janis Baird[2], Christina Black[1,2], Wendy Lawrence[2], Tannaze Tinati[1,2], Barrie Margetts[3], Hazel Inskip[2], Cyrus Cooper[2], Mary Barker[2]. *[1]NIHR Biomedical Research Unit Nutrition, Diet and Lifestyle, Southampton University Hospitals Trust, United Kingdom; [2]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom; [3]Institute of Human Nutrition, University of Southampton, United Kingdom.*
Maternal diet is the strongest predictor of a child's quality of diet at three years. However, it is not clear what factors underlie this association. Parenting control practices influence children's quality of diet. We explored the role of covert and overt control practices in the relationship between mothers' and children's diets. Overt control practices are those which can be detected by the child, covert practices cannot.
1022 women in Hampshire UK completed a food frequency questionnaire. A sub-sample of 348 women with 2-4 year old children also completed a food frequency questionnaire about their child's diet and their use of covert and overt controls over their child's eating habits. Principal components analysis produced a diet quality score for both mothers and children.
Mothers who had better quality diets tended to have children with better quality diets (p=<0.001), even after adjusting for mother's age, number of children, clothing size and educational attainment. Mothers' diet accounted for 32% of the variance in children's dietary quality. Mothers who used more covert, but not overt, controls over their children's eating habits also had children with better quality diets (p=<0.001). In a multiple regression model, maternal diet quality and covert control style were independent predictors of child's diet quality (p=<0.001 for both).
If a mother exerts covert control over her child's eating habits then the child is likely to have a better quality of diet. Parenting control practices over children's eating habits may be a focus for public health interventions to improve the quality of children's diets.

PII-251

Do Humans Prioritize Protein Intake? Claudia Martinez-Cordero[1], Christopher W. Kuzawa[2], Deborah M. Sloboda[1,3], Joanna Stewart[4], Stephen J. Simpson[5], David Raubenheimer[6]. *[1]The Liggins Institute, University of Auckland, New Zealand; [2]The National Research Centre for Growth and Development, New Zealand; [3]Department of Anthropology, Northwestern University, USA; [4]Biostatistics, University of Auckland, New Zealand; [5]School of Biological Sciences, University of Sydney, Australia; [6]Institute of Natural Sciences, Massey University, New Zealand.*
Protein has been identified as a key nutrient in determination of energy intake; a phenomenon termed Protein Leverage. The Protein Leverage Hypothesis (PLH) postulates that animals, including humans, prioritize protein intake over that of other macronutrients. PLH has been supported by experimental studies of macronutrient regulation and meta-analysis of experimental studies. We tested the hypothesis that energy intake from protein would remain more constant over time than energy intake from carbohydrates and fat in a population undergoing the nutrition transition. Data come from Filipino adult women participating in the Cebu Longitudinal Health and Nutrition Survey (CLHNS) from 1983 to 2005.
Longitudinal analyses were used to characterize changes in protein intake during the 22-year period (from 1983 to 2005). We analysed 24-hour dietary recall data to investigate differences in the change in macronutrient intake over time, and the effect of family income and urbanicity on macronutrient intake over the same period.
Energy intake from protein remained more constant over time than that from carbohydrates or fat in this female population undergoing the nutrition transition. Although energy intake from protein decreased over time, the decrease was smaller than that from carbohydrates while fat intake increased.
Our longitudinal data analysis indicates that energy intake from protein remained more constant than that from carbohydrates or fat in a human population undergoing the nutrition transition, even when family income and urbanicity change.

PII-252

The Therapeutic Effect of Plants on Rheumatoid Arthritis: Results from a Cross-Sectional Study in Sirajganj District of Bangladesh. Md. Ariful Haque Mollik, Romeo McField. *Health and Education, Practical Academy on Wise Education and Research Foundation, Bangladesh.*
Various types of body pains are common afflictions affecting people throughout the world. A more debilitating type of pain arises from rheumatoid arthritis, which is believed to affect at least 03% of the world's population, mostly the elderly. Since regular use or over-use of various pain-killer drugs may have side-effects, an alternative route to treat pain is through use of plants provided by the traditional health practitioners (THPs) and which are generally believed to be without any side-effects. The use of plants for treatment of rheumatoid arthritis varies considerably between different districts of Bangladesh. We accordingly conducted an ethnopharmacological survey amongst the THPs of Sirajganj district, Bangladesh to gather information on plants used to treat rheumatoid arthritis.
In-depth information regarding plants type, preparation of medicines, ailments for which they are used, dosages, and side effects if any, were obtained from the THPs. All plant samples were later identified at the Bangladesh National Herbarium.
The plant names used to treat rheumatoid arthritis included *Ocimum tenuiflorum* L., *Asparagus racemosus* Willd., *Sesamum indicum* L., *Zingiber officinale* Roscoe, *Cinnamomum tamala* (Buch.-Ham.) T.Nees & Eberm., *Cynodon dactylon* (L.) Pers., *Phoenix sylvestris* (L.) Roxb., *Cannabis sativa* L., *Hyptis suaveolens* (L.) Poit., *Cissus quadrangularis* L., *Cereus*

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grandiflorus (L.) Mill., *Cyperus scariosus* R.Br., *Allium sativum* L., *Tagetes erecta* L., *Lawsonia inermis* L., *Solanum rudepannum* Dunal, *Bambusa arundinacea* Willd., *Cinnamomum camphora* (L.) J.Presl, *Lens culinaris* Medik., *Piper nigrum* L., *Aerva sanguinolenta* (L.) Blume, *Morus alba* L., *Arachis hypogaea* L., *Musa × sapientum* L., *Nicotiana tabacum* L., *Clerodendrum indicum* (L.) Kuntze, *Brassica napus* L., *Nigella sativa* L., *Dysphania ambrosioides* (L.) Mosyakin & Clemants, *Aristolochia indica* L., *Hemidesmus indicus* (L.) R. Br. ex Schult., *Curcuma longa* L., *Azadirachta indica* A. Juss., *Withania somnifera* (L.) Dunal, *Persicaria vulgaris* Webb & Moq., and *Aphanamixis polystachya* (Wall.) R.Parker.

Overall, the patients of Sirajganj district appeared to be generally satisfied with the treatment offered through these plants, it is important to conduct proper scientific studies towards discovery of compounds of interest in these plants, which can be used as safe and effective medicines for rheumatoid arthritis.

PII-253

Programming Effects of Prenatal Stress and Betamethasone Exposure on the Activity of the Hypothalamic Pituitary Adrenal Axis (HPAA) in Relation to the Time of Exposure. Vilmar Frauendorf[3], Florian Rakers[1], Sven Rupprecht[1], Rene Schiffner[1], Harald Schubert[2], Sabine Bischoff[2], Matthias Schwab[1]. *[1]Dept.of Neurology, Friedrich Schiller University Jena, Germany; [2]Institute of Lab Animal Sciences, Friedrich Schiller University Jena, Germany; [3]Dept. of Hepatology and Gastroenterology, Charité Berlin, Germany.*

Epigenetic modifications mediated by prenatal stress (PS) and betamethasone (BM) exposure program hyperactivity of the HPAA. Though fetal HPAA doesn't become responsiveness before the end of gestation, stress early in gestation induces the most pronounced changes in stress-mediated behavioral responses during later live (Dodic, FASEB J, 2002).

Our objective was to examine (1) at which stage of pregnancy the HPAA is most vulnerable to stress, and (2) to compare the effects of PS with those of BM at the dose used to enhance lung maturation in babies threaten premature labor.

We used the sheep model in which BM therapy to enhance fetal lung maturation has been developed. 21 pregnant ewes were exposed to isolation stress twice weekly for 3h between 30 & 100dG (days gestation, term 150dG, early stress) or 100 & 121dG (late stress). 16 pregnant ewes received saline or 2x110µg/kg BM 24h apart at 106 1 and 112 1dG equivalent to 2x8 mg BM administered to a 70kg pregnant woman. Fetuses were instrumented at least three days before the 1st course of BM or the 1st test of HPAA activity in response to a hypotensive challenge using sodium nitroprusside at 112 and 129dG (i.e. before and during maturation of the fetal HPAA). Fetal serum cortisol was estimated using a RIA.

Basal cortisol levels were similar at 112 and 129dG in all groups. The hypotensive challenge led to an increase of fetal cortisol levels in all groups at 129 but not at 112dG ($p<0.05$). This increase was higher following PS and BM exposure than in controls ($p<0.05$). The increased rise in the cortisol response was similar during late PS and BM exposure but more pronounced following early PS ($p<0.05$). A parallel increase of ACTH suggests effects on central parts of the HPAA.

PS and BM treatment during the third trimester program hyperactivity of the HPAA in a similar way. PS during the first and second trimester had more pronounced effects suggesting high vulnerability of the fetal HPAA to epigenetic modifications early in gestation (i.e. at a time when the HPAA is still functionally inactive).

PII-254

Maternal Cortisol Shapes Fetal and Infant Development. Laura Glynn[1,2], Elysia Davis[1], Curt Sandman[1]. *[1]Psychiatry & Human Behavior, University of California, Irvine, CA, USA; [2]Crean School of Health and Life Sciences, Chapman University, CA, USA.*

To determine the association between exposures to prenatal maternal cortisol and fetal and infant reactivity to challenge.

Maternal cortisol levels (at 15, 19, 25, 31 and 36 weeks' gestation) and fetal movement response to vibroacoustic stimulation (VAS; at 25, 31 and 36 weeks) were assessed in 190 mother-fetus pairs. In addition, infant cortisol response to a heelstick at 24-hours of age was assessed.

Early elevations in cortisol predicted a failure to respond to the VAS at 25 weeks and later elevations were associated with a larger response to the VAS among term fetuses. Further, both maternal cortisol and the fetal response to VAS were predictive of physiological stress responding in the neonate.

The findings provide support for the role of prenatal glucocorticoids in shaping human CNS development.

PII-255

Infants' Cortisol Responses to the Still-Face Procedure: Stress Reactivity or Return to Baseline? Kerry-Ann Grant, Catherine McMahon. *Psychology, Macquarie University, New South Wales, Australia.*

The still-face paradigm (SFP) is being used with increasing frequency as a method for evaluating infant stress reactivity in the laboratory. However, since few studies include home baseline or control measures of cortisol activity it is unclear whether reported findings represent stress reactivity or simply a return to home baseline levels. In this study, we measured both home and laboratory baseline cortisol levels to examine whether the stress of the SFP is associated with cortisol reactivity in five month-old infants (M=5.2 months, SD=.89 months). A further novel aspect of the study was the assessment of individual differences in infant cortisol response. We tested the hypothesis that infant difficult temperament would be negatively associated with cortisol response to both the novelty of the testing environment (laboratory baseline) and the SFP.

Fifty-one mothers and infants (24 girls, 27 boys) completed a repeated SFP consisting of five, 2-minute episodes (play, still-face, play, still-face, play). Infant salivary cortisol levels were measured at baseline and at 10, 20 and 30 minutes following the SFP. The home sample was collected on a weekday and at a time that matched laboratory baseline sampling. Maternal report of infant temperament was assessed using the Short Temperament Scale for Infants, a widely used and reliable scale validated for use in Australian samples. Other variables known to impact laboratory baseline cortisol levels were also assessed (e.g., time of day, travel time to lab, time of last feed, time and length of last sleep).

Infant cortisol showed a significant increase over time in response to the SFP, $t(50) = -2.04$, $p < .05$. Although home cortisol levels (4.22 nmol/L) were higher than lab baseline levels (3.71 nmol/L), this difference was not significant, $t(50) = -1.09$, $p > .10$. Infant temperament was not associated with either laboratory baseline cortisol levels or with cortisol reactivity, p-values > .10. Baseline cortisol was unrelated to time of day, travel time to lab, time of last feed, time and length of last sleep.

Infant cortisol levels showed a significant increase from laboratory baseline following the SFP. Contrary to recent reports, infants' home baseline and laboratory baseline cortisol levels did not differ significantly. This suggests that the SFP provides a simple, laboratory-based psychological challenge that elicits at least a modest cortisol response that is not simply a return to baseline levels.

PII-256

The Effects of Prenatal Stress on the Intrauterine Growth of the Fetus. Titia Hompes[1], Elske Vrieze[1], Annelies Simons[2], Johan Verhaeghe[3], Karel Allegaert[4], Bea Van den Bergh[2], Koen Demyttenaere[1], Stephan Claes[1]. *[1]Psychiatry, University Hospitals Leuven, Belgium; [2]psychology, Tilburg University, Netherlands; [3]Gynaecology and obstretrics, University Hospitals Leuven, Belgium; [4]Paediatrics, University Hospitals Leuven, Belgium.*

We expected higher basal cortisol levels, or more depressive and anxious complaints during pregnancy, to be associated with a less IUG and lower birth weight.

Pregnant women (n=57) at eight to 12 weeks gestation were recruited from the antenatal clinic at the University Hospitals of Leuven, Belgium. Mothers were seen once during each trimester. Psychological assessments consisted of the Edingburgh Postnatal Depression Scale, Hospital Anxiety and Depression Scale, Pregnancy Related Anxiety Questionnaire and the Maternal Fetal Attachment Scale. The diurnal cortisol profile was derived from saliva samples. IUG was evaluated using ultrasound.

Statistical analysis was performed using SPSS17.0. In each trimester a multiple regression model was used to predict IUG measures and birth weight. To reduce the number of predictors in the regression models,

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principal component analyses (PCA) were performed on variables measured: anxiety, depression, attachment and cortisol ('area under the curve', AUC).
The second trimester predictors explained 20.2% (p=0.005) of the variance in birth weight; cortisol (AUC) was the only significant predictor (Portion of Variance Explained (PVE)=10.3%, p=0.008). 18.5% (p=0.000) of baby's BMI was explained by the second trimester predictors as well; the depression component was a significant predictor (PVE=7.3%; p=0.020).
In the third trimester model the predictors explained, 20.1% (p=0.017) of the variance in fetal growth (increase in estimated fetal weight between the 2nd and the 3rd trimester) and 12.0% (p=0.001) of the BMI at birth. Attachment turned out to be the only significant predictor in both cases PVE=16.3% (p=0.004) respectively PVE=6.5% (p=0.042).
The effects remained after controlling for relevant covariates (Gestational age at birth, sex of the baby, weight gain during pregnancy, smoking during pregnancy, parity and education) (p<0.05).
These data indicate that basal cortisol levels and maternal depression during second trimester influence birth weight and BMI, and thus fetal growth as such. During the third trimester, maternal attachment had an impact on further growth. Evidently, these are exploratory data in a relatively small sample size, and replication is needed.

PII-257

Effects of Maternal Anxiety during Pregnancy on Novelty Processing in Two-Month-Old Infants. An ERP Study. Renée A. Otte[1], Marijke A.K.A. Braeken[1], Bea R.H. Van den Bergh[1], István Winkler[2,3]. *[1]Department of Clinical and Developmental Psychology, Tilburg University, Netherlands; [2]Deparment of Experimental Psychology, Hungarian Academy of Sciences, Hungary; [3]Institute of Psychology, University of Szeged, Hungary.*
Research into the effects of prenatal maternal stress and anxiety found evidence for alterations in fetal neurodevelopment. Thus, there is need for precise neurophysiologic measures predicting cognitive development later in life. These measures should also be sensitive to individual differences in sensory-cognitive function induced by prenatal stress.
The present study tested in 2-month-old infants whether prenatal maternal anxiety affected the auditory mismatch negativity (MMN) event-related brain potential (ERP). MMN is a cortical response elicited by contextually deviant or novel sound events, reflecting how well infants can model the auditory environment and how strongly they react to novelty. Maternal state-anxiety was measured with the state-anxiety inventory (n=56) between the 8th and 14th week of pregnancy. Two months after birth, infants' ERPs were recorded during a passive auditory oddball paradigm delivering four types of sounds of 200ms duration at a uniform 300ms pre-stimulus interval: frequent standard complex tones (500Hz; p=70%), tones with 100ms pre-stimulus (p=10%), white noise segments (p=10%), and various environmental sounds (p=10%). Deviance/novelty-related responses were calculated by subtracting responses elicited by the standard tones separately from those elicited by each of the infrequent sounds.
Preliminary analysis shows that exposure to high levels of maternal state-anxiety during pregnancy tends to yield a smaller novelty response over most brain areas, except for the left-frontal areas, compared to those observed for infants exposed to normal or low levels of state-anxiety during pregnancy (F(8,46)=2,093;p=.056).
Sensory cognitive processes involved in detecting auditory deviance and novelty may be programmed differently for infants who in utero have been exposed to high versus low levels of maternal state-anxiety. These processes are important building blocks for passive attention and keeping track of the environment. Further research is needed, however, to establish how exposure to high maternal state-anxiety during pregnancy affects sensitivity to deviance and novelty.

PII-258

Transgenerational Predictors of Birth Weight in the Philippines: Correlation with Mother's and Father's Birth Weight and Test of Maternal Constraint. Christopher W. Kuzawa[1,2], Dan T. Eisenberg[1], M. Geoff Hayes[1]. *[1]Department of Anthropology, Northwestern University, IL, USA; [2]Cells 2 Society, Institute for Policy Research, Northwestern University, IL, USA.*
The contributions of genes and environmental influences to birth weight (BW), and to relationships between BW and later biology, remains a topic of debate. Past research has shown that maternal BW is a stronger predictor of offspring BW than is paternal BW, suggesting that maternal genetic and/or environmental factors play a disproportionate role in determining birth size. In addition, some but not all past studies have found that father-offspring BW correlations are stronger when the mother is taller, suggesting that small maternal size can constrain offspring size and override paternal genetic contributions. Here we report intergenerational BW correlations among females (n=709 births) and the spouses of study males (n=426 births) participating in a large longitudinal birth cohort study in Cebu City, the Philippines.
Recalled BW and gestational age among offspring of the now-adult birth cohort members was obtained by questionnaires administered to female participants and to spouses of male participants. Parental BW and gestational age were measured at baseline in 1984. Relationships between offspring and parental BW were evaluated using multiple regression and testing for significant differences in the strength of relationship by gender of parent.
After adjusting for potential confounding influences, a 1-kg change in mother's BW predicted a 246 ± 56 g increase in offspring BW (p<0.00001), while father's BW was a weaker predictor (beta = 124 ± 54 g, p<0.023). Consistent with the concept of maternal constraint, when BW of offspring born to spouses of male participants were stratified on tertiles of the mother's stature, there was a dose-response increase across height tertiles in the strength of the relationship linking paternal BW and offspring BW, with paternal BW only a significant predictor of offspring BW among men married to the tallest women.
Paternal genetic contributions to offspring BW are likely attenuated when the mother is shorter, perhaps indicating an overriding influence of maternal nutritional stress and stunting on offspring size. These relationships provide evidence for a maternal effect on offspring BW in Cebu, and underscore the likely importance of maternal environmental or genetic factors as an influence on offspring fetal growth rate.

PII-259

Antenatal Glucocorticoid Treatment and Learning and Memory in Offspring: Transgenerational Effects. Vasilis Moisiadis[1], Alisa Kostaki[1], Jeff Emack[1], Stephen G. Matthews[1,2,3]. *[1]Physiology, University of Toronto, ON, Canada; [2]Obstetrics and Gynaecology, University of Toronto, ON, Canada; [3]Medicine, University of Toronto, ON, Canada.*
Approximately 10% of pregnant women are at risk of preterm delivery and receive synthetic glucocorticoids (sGCs) to reduce the risk of infant respiratory distress syndrome. We have shown that prenatal sGC exposure alters stress responsiveness and locomotor activity in first (F_1) and second (F_2) generation offspring. Increased locomotor activity in F_1 offspring is associated with altered hippocampal NMDA receptor expression and hippocampal long-term potentiation (LTP). In the present study, we hypothesized that maternal exposure to sGC results in impaired learning and memory in F_1 and F_2 offspring.
Pregnant guinea pigs (F_0; n=8-10/gp) were subcutaneously injected with betamethasone (BETA; 1mg/kg) or vehicle (VEH; saline) on gestational days 40 & 41, 50 & 51 and 60 & 61 to generate F_1 offspring. Subsequently, adult F_1 female offspring from each treatment group (n=7-8/gp) were mated with control males to generate F_2 offspring. F_1 and F_2 male and female offspring were tested in the Morris Water Maze to assess spatial learning and memory on postnatal days 35 (juvenile) and 70 (adult). Latency to find a hidden platform, retention of platform location and search strategy were analyzed.
All groups effectively learnt the location of the hidden platform. However, there was no effect of prenatal (F_0) sGC exposure on latency to find the platform in juvenile or adult offspring in either generation. There were also no significant differences in memory of the platform location (probe trial)

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between any of the groups. However, both juvenile and adult female F_1 offspring whose mothers received sGCs during pregnancy used a different strategy to search for the platform's location during the probe trial ($p<0.05$), indicating a greater ability to adapt to changing conditions (i.e. removal of the platform during the probe trial).

In conclusion, while antenatal treatment with sGCs has strong transgenerational effects on locomotor activity and neuroendocrine function, there appears to be little effect of this treatment on measures of learning or memory. Together, these results suggest that the processes of memory and learning are resilient to the effects of prenatal exposure to sGCs.

PII-260

Bourne Back Ceaselessly into the Past: Policy Initiatives To Address the Intergenerational Implications of Prenatal and Postnatal Marginalization. Valerie A. Pacino. *Community-Oriented Public Health Practice, University of Washington, WA, USA.*

This investigation aims to detail models of health, examine the implications of historical trauma and an ecology of fear on marginalized communities, and link DOHaD theories to policy initiatives.

A literature review reveals that too few scientists and researchers are willing to examine the implications of historical trauma and an ecology of fear. While the genetic and behavioral models of health have historically commanded the attention of researchers, practitioners, clinicians, the media, and the public, DOHaD theorists have rejected these as the primary drivers of health. The socioeconomic status paradigm has gained traction over the last several years, but still fails to account for many heretofore unknown determinants of health. Environmental justice models insist that health begins where we live, work, play, and learn.

When community-based participatory researchers have exhibited the boldness required to examine the ecology of fear within marginalized communities, they are rewarded with staggering answers about the intergenerational implications of structural violence. An understanding of the social determinants of health has clarified the life-long influence of prenatal and postnatal environments. For at least the last century, the fetus was believed to be impervious to the stressors and toxins to which its mother was exposed; rather than founded on science, this reflected a deeply patriarchal effort to define medicine against midwifery and devastate local, place-based knowledge and women's reproductive rights. Not only does the prenatal environment matter tremendously for infant health outcomes, the epigenetic legacy of a human's first days, months, and years matters for child and adult health outcomes, as well. Furthermore, the intergenerational transmission of prenatal and postnatal marginalization appears to be an evolutionary adaptation that has devastated specific communities and advantaged others.

Public health is poised on the crest of a great wave, and its comprehensive political and social responses to the developmental origins of health and disease matters tremendously. Possible policy changes that should be considered include paid prenatal and postnatal parental leave, free compulsory programs for kids 0-5 and their families, a living wage, and a maximum wage.

PII-261

Intergenerational Associations between Maternal Height and Cardiovascular Disease Risk in the Offspring – Findings from the New Delhi Birth Cohort. Poornima Prabhakaran[1], Dimple Kondal[1,2], H.P.S. Sachdev[3], Santosh Bhargava[4]. *[1]Public Health Foundation of India, New Delhi, India; [2]Centre for Chronic Disease Control, New Delhi, India; [3]Sitaram Bhartia Institute of Science and Research, New Delhi, India; [4]Sunderlal Jain Hospital, New Delhi, India.*

Background and Objectives – Extensive research in developed countries has shown that parental influences on cardiovascular disease risk in their children can be linked back to their own earlier nutritional or socio-economic circumstances. Adult height is a sensitive marker of early life circumstances and evidence from developing countries is sparse in this area. We examined the association of maternal height with risk of offspring cardiovascular disease (CVD) in the New Delhi Birth Cohort.

Methods – 8,181 singleton live births to married women born between1969-72 followed up in New Delhi. Anthropometry was prospectively recorded at birth and 6-monthly until 14-21 years. Metabolic risk factors were measured in 1,526 as adults in 1998-2002. We examined the association of maternal height with cardiovascular disease risk in cohort members using multiple regression analyses.CVD risk factors included waist circumference, skin folds, body mass index, fasting, 30-minute and 2-h glucose, insulin, HOMA-Insulin Resistance, lipid profile and blood pressure.

Results – Maternal height was significantly associated with waist circumference [β (SE) =0.2800(0.0749), 95% CI= (0.1329, 0.4270)], fasting glucose [β (SE) =0.3569(0.1326), 95% CI= (0.0967, 0.6172)] and blood glucose at 30 minutes [β (SE) =0.6452 (0.2275), 95% CI= (0.1987, 1.092)]. ($p<0.0001$), after adjusting for offspring age and sex. The associations remained significant even after adjusting for socio-economic status of parents at childbirth as well as current socio-economic status of the cohort members. For two hour blood glucose, the association was not statistically significant .There were no statistically significant associations with other CVD risk factors as well.

Conclusions – Maternal height in this population is associated with increase in some cardiovascular disease risk factors in their offspring. Increasing affluence and changing socio-environmental factors with associated changes in lifestyle and habits may be the reason for these findings and may be explored further.

PII-262

Length and Weight Gain in Infancy as Predictors of Birth Weight in Next-Generation. H.P.S. Sachdev[1], C. Osmond[2], C.H.D.F. Fall[2], S.K. Bhargava[3]. *[1]Sitaram Bhartia Institute of Science and Research, New Delhi, India; [2]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom; [3]Sunder Lal Jain Hospital, Delhi, India.*

Is increased growth in infancy associated with next-generation birth weight in low and middle income countries? If so, then what is the relative importance of length and weight gain?

510 men and women aged 36 years from a longitudinal study that started in New Delhi, India, in 1969-1972. Anthropometry was prospectively recorded at birth and 6-monthly until 14-21 years, in 1998-2002 (30 years) and in 2006-2009 (36 years). Next generation birth weight was available from medical records in 870 children (510 first born and 360 later births).

We describe a statistical approach to distinguish linear (skeletal) growth from weight gain (mainly soft tissue growth) in early infancy (birth to six months) and late infancy (six months to two years), and use it to predict next generation birth weight. "Conditional height gain" is height gain in an interval beyond that which would have been predicted from all measurements of height and weight known at the beginning of the interval. "Conditional weight gain" is defined similarly, but also includes height at the end of the interval in the prediction. Independent variables adjusted for in multivariate mixed model included child and parent gender, birth order, social class at birth and adulthood, birth weight and length, the four conditionals as above, and adult length and weight (of parents).

Conditional gain in both length and weight at six months predicted next-generation birth weight. This significant association was evident for both fathers and mothers. The association was marginally stronger ($p>0.05$) for conditional weight gain. There was an average difference of adjusted birth weight of 207 grams between first and fifth quintiles for conditional weight gain; the corresponding value for conditional length gain was 77 grams. Conditional gain in length or weight at two years did not predict next-generation birth weight.

Weight gain in early infancy (over and above birth size and linear growth) and length gain in early infancy (over and above birth size) are associated with increased birth weight in next generation. These data support the need to promote early infant growth in low and middle income countries like India.

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Maternal Pre-Pregnancy Body Mass Index and Risk for Affective Disorders in Offspring: A Prospective Pregnancy Cohort Followed to Late Adolescence. Monique Robinson[1], Stephen R. Zubrick[1], Craig E. Pennell[1], Ryan J. Van Lieshout[2], Peter Jacoby[1], Lawrence J. Beilin[1], Trevor Mori[1], Fiona J. Stanley[1], John P. Newnham[1], Wendy H. Oddy[1]. *[1]The University of Western Australia, Australia; [2]McMaster University, Canada.*

Maternal pre-pregnancy obesity has been linked with an increased risk for negative emotionality and inattentiveness in offspring in early childhood. The aim of this study was to examine the association between maternal pre-pregnancy body mass index (BMI) and the development of affective problems (dysthymic disorder, major depressive disorder) throughout childhood and adolescence in a prospective pregnancy cohort.

In the Western Australian Pregnancy Cohort (Raine) Study, 2,900 women provided data on their pre-pregnancy weight, and height measurements were taken at 18 weeks gestation. BMI was calculated and categorized using World Health Organization cutoffs (2004). Live born children were followed up at ages five, eight, ten, 14 and 17 years and the DSM-oriented scales of the Child Behaviour Checklist (CBCL) were used to obtain affective problems scores at these ages. At age 17 years the study adolescents also completed the Beck Depression Inventory for Youth (BDI-Y). Longitudinal mixed models and generalized estimating equations were applied to assess relationships between maternal pre-pregnancy BMI and affective problems from age five through 17 years. Separate models were used to examine associations between maternal pre-pregnancy BMI and BDI-Y scores at 17 years with adjustment for adolescent BMI.

There was a higher risk of affective problems between the ages of five and 17 years among children of women who were obese (OR= 1.57, 95% CI= 1.05, 2.35), compared with the offspring of women in the healthy pre-pregnancy weight range (BMI 18.5-24.99) and after adjustment for confounders. This relationship was further supported by significantly increased scores on the BDI-Y scores at age 17 years (b= 3.38, 95% CI= 0.77, 6.00) in offspring of obese mothers. Given our extensive adjustment for sociodemographic factors and perinatal complications, we suggest our results are not due to residual confounding.

Maternal pre-pregnancy obesity may be implicated in the development of affective problems, including depression, in their offspring later in life.

PIII-264

Are Infants Born at 37 Weeks Gestation at Increased Risk for Behavioral Problems through to Adulthood? Monique Robinson, Stephen R. Zubrick, Andrew J.O. Whitehouse, Craig E. Pennell, Peter Jacoby, Wendy H. Oddy, Fiona J. Stanley, John P. Newnham. *The University of Western Australia, Australia.*

Recent debate questions the suitability of 37 weeks gestational age as a demarcation of high versus low risk for ongoing optimal development of the child. The aim of this study was to examine the behavioral sequelae for children born at 37 weeks gestation in comparison with those born preterm (<34 weeks), late preterm (34-36 weeks) and at 38 and 39 or more weeks gestation.

The Western Australian Pregnancy Cohort (Raine) Study provided comprehensive obstetric data from 2,900 pregnancies resulting in 2,868 live born infants. Children were followed up at ages 2, 5, 8, 10, 14 and 17 years using the Child Behavior Checklist (CBCL) with clinical cutoffs for behavioral morbidity for overall, internalizing (withdrawn, somatic, anxious/depressed) and externalizing (delinquent, aggressive) behavior (T≥60). We used longitudinal regression models with generalized estimating equations (GEE) with adjustment for maternal sociodemographic information from the prenatal period (maternal age, education, family income and structure and maternal smoking) along with perinatal data including birthweight, gestational hypertension and labor onset (nil (C-section), spontaneous or induced).

Approximately 9% of our cohort was born within the range of 37 0/7 and 37 6/7 weeks. Over 17 years, being born at 37 weeks gestation was associated with an increased risk for total (OR=1.54, 95%CI= 1.06, 2.23) and externalizing OR=1.47, 95%CI= 1.00, 2.16) behavioral problems in the fully-adjusted model when compared with infants born from 39 weeks. Infants born late preterm (34-36 weeks) and at 38 weeks did not show a significantly increased risk for behavioral problems in comparison with those born from 39 weeks onwards.

Our findings indicate that infants born at 37 weeks are at increased risk for behavioral problems over childhood and adolescence compared to those born later. We suggest that 37 weeks may not be the optimal cutoff for defining perinatal risk for behavioral development, although we accept that further exploration of the complications of pregnancy leading to birth at 37 weeks is warranted. A potential explanation of our findings is that infants born at 37 weeks are currently not targeted for behavioral support in the same way that infants born late preterm are, and this may have implications for policy regarding obstetric risk management and ongoing child development.

PIII-265

Epigenetic Inheritance of Anxiety-Like Behaviour in Rats – Role of Early Life Exposure to a Bacterial Mimetic. Luba Sominsky[1], Adam K. Walker[1], Lin K. Ong[2], Ross J. Tynan[2,3,4], Frederick R. Walker[2,3,4], Deborah M. Hodgson[1]. *[1]School of Psychology, The University of Newcastle, NSW, Australia; [2]School of Biomedical Sciences & Pharmacy, The University of Newcastle, NSW, Australia; [3]Priority Research Centre for Brain and Mental Health Research, The University of Newcastle, Australia; [4]Hunter Medical Research Institute, Australia.*

Postnatal exposure to a bacterial mimetic, lipopolysacharide (LPS) is known to produce long-lasting behavioural alterations, such as anxiety as well as long-term alterations in metabolic, neuroendocrine, immune and reproductive functioning. We have previously demonstrated that rats postnatally exposed to LPS exhibit increased anxiety-like behaviour in adulthood. These behavioural changes are typically associated with neuroendocrine and immune alterations. Furthermore, these changes were shown to persist into subsequent generations. Epigenetic modifications through chromatin remodelling have been previously suggested to underlie the effects of early life trauma on later life development. Therefore our current aim was to examine possible central mediators responsible for this transgenerational phenomenon.

Wistar rats were administered either LPS (0.05 mg/kg, ip) or non-pyrogenic saline on days three and five postpartum. In adulthood (day 85) anxiety-like behaviour was assessed on the Elevated Plus Maze and Holeboard apparatuses, following which animals were perfused, brain tissue collected and immunohistochemically stained for global assessment of microglial activation and histone H3 acetylation in the hippocampus and basolateral amygdala. Immediate effect of the immune challenge was determined in a subset of pups by assessment of plasma corticosterone and tyrosine hydroxylase (TH) phosphorylation in the adrenal medulla.

LPS exposure resulted in a significant increase in plasma corticosterone and TH phosphorylation four and 24 hours following LPS administration on postnatal day 5 ($p<.05$). In adulthood, LPS-treated rats exhibited significantly increased anxiety-like behavior, increased hippocampal microglial activation and histone H3 acetylation compared to saline-treated controls ($p<.05$ for all). Microglial activation and histone H3 acetylation were not colocalized in any of the regions assessed.

These findings suggest two central pathways through which neonatal LPS exposure can produce behavioural and neuroendocrine changes that persist into adulthood.

PIII-266

Developmental Programming of Cardiac Dysfunction Arising from Maternal Obesity; Potentiation by an Obesogenic Postnatal Diet. Ashleigh J. Haken[1], Chadni K. Rajani[1], Anne-maj Samuelsson[1], James Clark[2], Micheal Shattock[2], Lucilla Poston[1], Paul D. Taylor[1]. *[1]Division of Womens Health, King's College London and King's Health Partners, United Kingdom; [2]Cardiovascular Division, King's College London and King's Health Partners, United Kingdom.*

We have recently reported a novel murine model of developmental programming secondary to diet-induced maternal obesity. Offspring of obese dams (OffOb) exhibit hyperphagia, excess adiposity, insulin resistance and hypertension (Samuelsson *et al.*, 2008). Neonates from obese dams showed evidence of cardiac remodelling with increased heart weight and histological evidence of hypertrophy and hyperplasia. In this study we investigate the

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functional impact of early cardiac remodelling in offspring of obese mice (OffOb) using small animal high frequency ultrasound and the potential interaction with a post natal obesogenic diet.

Female C57BL/6J mice were fed either a standard chow diet (3% fat, 7% sugars) or a highly palatable, obesogenic diet (16% fat, 33% sugars) for six weeks prior to mating and throughout pregnancy and lactation. Offspring were either weaned onto standard chow (SC) or the same obesogenic diet as the dams (HFD). At six months, Small Animal Micro-Echocardiography Imaging was performed employing the Vevo 770® v1.2, with a RMV 707B scanhead (Visualsonics, Canada).

All mice exposed to the Ob diet post-natally demonstrated an increased heart weight and a reduced heart rate when corrected for body weight, compared to controls (P<0.0001). Micro-Echocardiography Imaging , revealed left ventricular posterior wall thickening (LVPW OffCon versus OffOb P=0.02) and thickened intra-ventricular septum (IVS, OffCon versus OffOb, P=0.02) in both males and females OffOb. Additionally, the high fat postnatal diet increased LVPW and left ventricle anterior wall (LVAW) thickness significantly (LVAW OffCon versus OffOb, P =0.04), and increased heart rate in OffOb compared to OffCon (P=0.01). The ventricular volume did not decrease in size significantly, indicating dilation of the hearts in obese animals.

Maternal obesity to increases cardiac wall thickening in both male and female offspring. Several aspects of cardiac remodelling in the OffOb mice are potentiated, by an obesogenic postnatal environment. Evidence of cardiac dilation supports the developmental programming of a heart failure phenotype secondary to maternal obesity.

Samuelsson, A.M., *et al.* Hypertension, 2008. **51**(2): p. 383-92.

PIII-267

Temporal Changes in Mean Arterial Pressure and Components of Vascular Renin Angiotensin System in Male and Female Offspring of Protein Restricted Dams. K. Sathishkumar, Meena Balakrishnan, Haijun Gao, Chandra Yallampalli. *Ob/Gyn, University of Texas Medical Branch, TX, USA.*

Protein restriction during gestation in the rat results in intrauterine growth restriction (IUGR) and development of hypertension during adult life. Numerous reports suggest that renin angiotensin system (RAS), a regulatory system important in the long-term control of blood pressure, is altered by maternal protein restriction and may contribute to the etiology of IUGR hypertension. However, the question of whether alterations in RAS components are a cause or consequence of hypertension development remains unanswered. Therefore, the purposes of this study is to determine whether temporal changes in RAS expression in vasculature are observed in IUGR offspring and to verify whether mesenteric vascular responses to angiotensin II (Ang II) are altered in adult offspring.

Male and female offspring generated from pregnant rats fed with protein restricted (PR; 6% casein) and control (C; 20% casein) diet were used. Progressive changes in the mean arterial pressure (MAP) and mRNA transcripts for RAS components (AT1R, AT2R, ACE and ACE2) in the mesenteric artery at 1, 3, and 6 months of age, and mesenteric vascular response to Ang II at six months of age were examined.

In the male offspring, MAP was similar between groups at one month of age but was significantly higher at three and six months of age in the PR compared with the C group. The vascular ACE/ACE2 ratio was significantly higher at one month of age while AT1R/AT2R ratio was higher at three and six months of age in PR compared with the C group. In the female offspring, MAP was similar between groups at one and three month of age but was significantly higher at six month of age in the PR compared to C group. Vascular ACE/ACE2 ratio and AT1R/AT2R ratio was significantly higher only at six month of age in PR compared to C group. ANG II-induced contractile responses in mesenteric arterial rings were increased in the PR group with more pronounced effect in the males than in the females compared to their respective C group. Responses to serotonin and phenylephrine were similar between groups at six months of age.

Temporal alterations in vascular RAS and exaggerated vasoconstriction to ANG II appear to correlate with the progression and degree of hypertension in the male and female IUGR offspring of protein-restricted dams. Therefore, RAS may play a key role in the etiology of IUGR hypertension.

PIII-268

Testosterone Contributes to Elevations in Mean Arterial Pressure in Adult Intra Uterine Growth Restricted Male and Female Offspring. K. Sathishkumar, Chandra Yallampalli. *Ob/Gyn, University of Texas Medical Branch, TX, USA.*

Elevated testosterone levels during pregnancy results in fetal growth restriction and programs for endocrine dysfunction with alteration in sex steroid hormone levels and gender-related hypertension in the adult male and female offspring. In this study we characterized whether alteration in sex steroid hormone levels contributes to the development of hypertension in the adult male and female offspring exposed to elevated androgens during late gestation.

Sprague-Dawley rats were injected daily with testosterone propionate (TP) @ 0.5 mg/kg or vehicle from gestational day 15-19. This dose of TP produces a 2-fold increase in circulating maternal testosterone levels similar to that observed in pregnant women complicated with intrauterine growth restriction. The male and female offspring of both control and testosterone administered dams were divided into three groups at seven weeks of age: sham surgery, gonadectomy and gonadectomy with hormone replacement (testosterone for males and estradiol for females). At 16 weeks of age, mean arterial pressure (MAP) and plasma estradiol and testosterone levels were measured.

The male and female offspring were smaller at birth by 11% and 14% respectively compared to corresponding controls. In the male offspring, MAP was significantly higher in TP (122±5 mmHg) compared to control (100±4 mmHg) offspring. Orchiectomy significantly reduced MAP in TP offspring (104±3 mmHg) but had no effect in controls (97±5 mmHg). Testosterone replacement recapitulated hypertension in the TP offspring (129±4 mmHg) but was without significant effect in controls (105±4 mmHg). In the female offspring, MAP was significantly higher in TP (111±5 mmHg) compared to control (94±3 mmHg) offspring. Ovariectomy significantly reduced MAP in TP offspring (97±2 mmHg) but had no effect in controls (92±3 mmHg). Estradiol replacement in ovariectomized rats did not significantly alter MAP in the TP offspring (99±4 mmHg) and controls (92±4 mmHg). Plasma testosterone levels were significantly higher in male and female TP offspring with intact gonad that was reversed by gonadectomy. Plasma estradiol levels were not different between groups.

Based on these findings and our previous report of androgen receptor antagonist reversing programmed hypertension in female offspring of protein restricted dams suggest that testosterone may play an important role in development and maintenance of hypertension of developmental origin.

PIII-269

Glucose Regulation in Embryonic Heart Development. Devon Scott[1], Sandra Rugonyi[1], Kent Thornburg[2], Monica Hinds[1]. *[1]Biomedical Engineering Department, Oregon Health Science University, OR, USA; [2]Heart Research Center, Oregon Health Science University, OR, USA.*

The prevalence of diabetes is rising in the population; maternal diabetes affects 7% of pregnancies resulting in a 3-5 fold increased risk for fetal cardiac and valve malformations. Elevated maternal blood glucose levels facilitate maternofetal diffusion through the placenta, which elevates fetal glucose levels and leads to a hyperinsulinemic state. The combination of hyperglycemia and hyperinsulinemia is toxic to endocardial cells (ECs), and may alter their response to changes in hemodynamic conditions resulting in the suppression of the mechanical signals required for normal development. While high maternal glucose levels are associated with heart defects, the effect of maternal diabetes on early fetal heart development has been little studied. In this project we aim to determine the degree to which a spike in glucose concentration alters the expression of EC shear sensitive genes in an embryonic heart. The EC specific genes targeted in this study, ET-1, eNOS, TGF-β, E-Cadherin and KLF-2, have been shown to be regulated by hyperglycemia or hemodynamics and identified as critical factors in heart and valve development.

To study the influence of altered glucose on ECs, the outflow tract (OFT) of embryonic chick hearts were studied at Hamburger Hamilton (HH) stage 24, ~day 4 incubation. Multiple single doses of glucose or control (vehicle) were administered near the embryo daily over the four day embryonic incubation time. Embryos were removed from the egg and the embryonic OFT were extracted for either qPCR or immuno-fluorescence staining.

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A single exogenous glucose dose given to the embryo led to a glucose spike with maximal glucose-plasma concentration at 60 minutes followed by a return to normal by 120 minutes. Daily glucose additions resulted in a stage delay of six hours over the four days and altered EC gene expression in the in vivo OFT (e.g., 3.5-fold increase in ET-1 and 3.5-fold decrease in E-Cadherin) compared to OFT controls during early stages of development.
We determined that spikes in circulating plasma glucose levels affect the function of ECs in the OFT of embryonic chick hearts. This study may elucidate mechanism by which glucose is toxic in the developing embryo.

PIII-270

Aortic Stiffening in Low Birth Weight Offspring Linked to Persistent Changes in Cellular Phenotype. Jennifer A. Thompson[1,2], Rob Gros[1], Bryan S. Richardson[1,2], Timothy R.H. Regnault[1,2]. *[1]Physiology and Pharmacology, The University of Western Ontario, ON, Canada; [2]Children's Health Research Institute, ON, Canada.*
Central arterial compliance is a powerful predictor of cardiovascular disease and primarily a function of the matrix components, elastin and collagen. Over the second half of gestation, these proteins are rapidly deposited by synthetic-type vascular smooth muscle cells (VSMCs) that undergo phenotypic modulation into contractile cells. De-differentiation of mature VSMCs to their synthetic precursors contributes importantly to pathological remodeling in postnatal life. We aimed to determine the cellular, structural and mechanical characteristics of the aorta in fetuses and offspring that were growth restricted *in utero*.
Placental insufficiency was induced in pregnant guinea pigs at mid-gestation by uterine artery ligation. Appropriate-for-gestational age (AGA) and small-for-gestational age (SGA) groups were defined according to relative fetal size in relation to the total population. In aortic cross-sections immunofluorescence was applied to measure the % area stained for non-muscle myosin heavy chain (MHC-B), a marker for synthetic-type VSMCs, and the contractile protein, α-actin. Collagen and elastic fibre content were analyzed using Sirius Red and Orcein staining, respectively. The area stained was expressed relative to the area non-stained and total content was calculated by multiplying the average wall thickness by the % area stained. Length-tension relationships were generated in aortic rings from adult offspring.
The % area stained for MHC-B within the aortic media was 6-fold higher in SGA (n =12) compared to AGA (n = 8) fetuses ($p < .0001$) and 3-fold higher in SGA (n = 7) versus AGA (n = 5) adult offspring ($p < .05$). The increase in MHC-B in SGA offspring concurred with a 41% increase in total collagen content and a 33% and 56% increase in relative and total α-actin content, respectively ($p < .05$). Relative elastic fibre content was decreased by 10% in SGA fetuses. This difference in relative elastic fibre content between SGA and AGA animals in adulthood was 2-fold and the total number of elastic laminae adjusted for wall thickness was 25% lower in SGA vs. AGA offspring ($p < .01$).
Retention of synthetic properties of VSMCs magnify the reduced elastic fibre content established *in utero*, leading to aortic stiffening in growth restricted offspring.

PIII-271

Left Ventricular Isovolumic Relaxation in Human Embryo: Relationship with Cardiac Afterload in Pre- and Postnatal Hypertension. Pavel B. Tsyvian[1,2,3], Vladislav V. Kovalev[1,2], Olga P. Kovtun[1]. *[1]Ural State Medical Academy, Yekaterinburg, Russian Federation; [2]Biophysical Lab, Mother and Child Institute, Yekaterinburg, Russian Federation; [3]Biomechanics, Institute of Immunology and Physiology, Yekaterinburg, Russian Federation.*
First trimester nuchal translucency (NT) and ductus venosus reverse blood flow (DVRBF) are used as ultrasound markers (UM) to determine the risk of fetal aneuploidy, heart defects and subsequent maternal preeclampsia. One pathophysiological explanation for these UM, encompassing both normal and pathological outcomes, is the development of transient heart failure due to increased vascular resistance (afterload). Left ventricular isovolumic relaxation time (LV IRT) was demonstrated as sensitive index of afterload increase in the second and third trimester fetus. The objective of study was to determine LV IRT in normally developing embryos and embryos with early UM of chromosomal and cardiac abnormalities and to test the hypothesis of embryonic hypertension as a cause of transient heart failure.
An ultrasound study (Philips HD 11) in 122 normally developing and 27 human embryos with increased NT (>3mm) and DVRBF at gestational ages 11-14 weeks. On the Doppler waveform traces the LV IRT (ms) was determined from the artifact of aortic wave closure to the onset of transmitral flow.
Mean LV IRT (41±3 ms) was 36.6 percent longer in embryos with UM as compared to the normal subset (30±2 ms) ($p<0.001$). Heart rate has no significant effect on LV IRT in both groups of embryos.
Earlier we have shown that LV IRT was significantly (29%) increased in the growth retarded (GR) fetuses which may herald raised systolic blood pressure in the early neonatal period [Tsyvian e.a. Eur.J.Obstet.Gynecol.Reprod. Biol.2008, 140:33-37]. Considerable reduction in vascular endothelial growth factors (VEGF) synthesis was demonstrated in embryos with UM of chromosomal, cardiac abnormalities and subsequent development of maternal preeclampsia. We speculate that the disturbances in VEGRF synthesis may cause some delay in the development of the vascular bed in comparison with heart development and a transitory increase in afterload in the 11-14 weeks embryo. Our observations support the hypothesis that transient heart failure due to increased afterload may be one of the causes of NT and DVRBF in embryo. We suggest that increase in embryonic LV IRT may precede arterial hypertension in the growth retarded fetuses during the second and third trimester.

PIII-272

Cardiovascular Consequences of Famine in the Young. Annet F.M. van Abeelen[1,2], Sjoerd G. Elias[1], Patrick M.M. Bossuyt[2], Diederick E. Grobbee[1], Yvonne T. van der Schouw[1], Tessa J. Roseboom[2,3], Cuno S.P.M. Uiterwaal[1]. *[1]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, Netherlands; [2]Department of Clinical Epidemiology, Biostatistics, and Bioinformatics, Academic Medical Center, Amsterdam, Netherlands; [3]Department of Obstetrics and Gynecology, Academic Medical Center, Amsterdam, Netherlands.*
The developmental origins hypothesis proposes that undernutrition during fetal life, infancy, or childhood is associated with an increased risk of cardiovascular disease in adulthood. As data on postnatal developmental programming are scarce, we investigated whether exposure to undernutrition during childhood, adolescence, or young adulthood is related to coronary heart disease (CHD) in adult life.
We studied 7,845 women from the Prospect-EPIC cohort who had been exposed at various degrees to the 1944-1945 Dutch Famine when they were aged between 0 to 21 years. These women had reported individual levels of exposure to hunger during the famine. We used Cox proportional hazard regression models to explore the effect of famine on the risk of CHD, overall and within exposure age categories (0-9, 10-17, ≥18 years). We adjusted for potential confounders, including age at famine exposure, smoking, and level of education as a proxy for socio-economic status.
Overall, stronger famine exposure was associated with higher CHD risk. Among those who experienced the famine between ages 10 to 17 years, CHD risk was significantly higher among severely famine exposed women compared to unexposed women (HR 1.38, 95% CI 1.03 to 1.84).
Exposure to undernutrition during postnatal periods of development, including adolescence, affects the risk of cardiovascular disease in adult life.

PIII-273

Do Both IGF-1R and IGF-2R Signalling Increase Cell Area in Cultured Fetal Sheep Cardiomyocytes? Kimberley C.W. Wang[1], Doug A. Brooks[2], Kimberley J. Botting[1], Janna L. Morrison[1]. *[1]Early Origins of Adult Health Research Group, School of Pharmacy and Medical Sciences, University of South Australia, South Australia, Australia; [2]Mechanisms in Cell Biology and Diseases Research Group, School of Pharmacy and Medical Sciences, University of South Australia, South Australia, Australia.*
In vitro rat experiments have shown that IGF-2R activation can induce cardiomyocyte hypertrophy via a G protein coupled receptor (Gαq)-dependent manner but, it is not known if this pathway exists in the fetal sheep heart. We therefore aimed to investigate if IGF-1R increases cell area

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in cultured fetal sheep cardiomyocytes. To determine if IGF-2R activates downstream signalling proteins of Gαq to increase fetal sheep cultured cardiomyocytes cell area.

Ewes were humanely killed at 126d ±1d gestation and fetuses were delivered by hysterotomy. Hearts were dissected, dissociated and the isolated cardiomyocytes were cultured. Cultured cardiomyocytes were treated with either LONG™R^{3}IGF-1, an agonist of IGF-1R; Picropodophyllin (PPP), an IGF-1R inhibitor; Leu^{27}IGF-2, an IGF-2R agonist; KN-93, an inhibitor of Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII) or Gö6976, a *protein kinase C* (PKC) isotypes (α, β and γ) inhibitor. For cardiomyocytes that were exposed to both inhibitor and agonist, the inhibitor was added 30 min prior to the addition of the agonist. Cultured cardiomyocytes were stained with anti-myosin to enable the measurements of binucleated cardiomyocyte cell area that were expressed relative to the serum-free culture medium control for the respective animal.

LONG™R^{3}IGF-1 treatment increased binucleated cell area while inhibition of IGF-1R with PPP resulted in reduced cell area of binucleated cardiomyocyte. Relative binucleated cell area remained reduced when cells were treated with both LONG™R^{3}IGF-1 with PPP. Leu^{27}IGF-2 increased the area of cultured binucleated cardiomyocytes. Inhibition of PKC with Gö6976 did not change the cell area of binucleated cardiomyocyte; however, inhibition of CaMKII with KN-93 resulted in reduced cell area of binucleated cardiomyocyte. Inhibition of CamKII continued to reduce cell area in the presence of both Leu^{27}IGF-2 and KN-93.

Our results indicate that stimulation of IGF-1R via IGF-1 increases cultured cell area of binucleated cardiomyocyte. Independently, activation of IGF-2R increases cell area in cultured fetal sheep binucleated cardiomyocytes via CaMKII.

PIII-274

Activation of IGF-2R Stimulates Hypertrophy in Fetal Sheep Heart. Kimberley C.W. Wang[1], Doug A. Brooks[2], Kent L. Thornburg[3], Janna L. Morrison[1]. *[1]Early Origins of Adult Health Research Group, School of Pharmacy and Medical Sciences, University of South Australia, South Australia, Australia; [2]Mechanisms in Cell Biology and Diseases Research Group, School of Pharmacy and Medical Sciences, University of South Australia, South Australia, Australia; [3]Heart Research Center, Oregon Health Science University, OR, USA.*

In vitro rat studies indicate that insulin-like growth factor 2 receptor (IGF-2R) has the ability to induce cardiomyocyte hypertrophy via a G protein coupled receptor (Gαq)-dependent manner in the heart. However, it is not known if IGF-2R can induce cardiomyocyte proliferation or hypertrophy the heart during fetal life. We therefore aimed to determine if activation of IGF-2R leads to cardiac proliferation or hypertrophy *in vivo* in the sheep fetus in late gestation.

Fetuses underwent surgery where catheters were inserted into the carotid and left circumflex coronary arteries, trachea and amniotic cavity. Leu^{27}IGF-2, an IGF-2R agonist, was then infused via the left circumflex coronary artery for four days with carotid blood pressure recorded throughout the period. Ewes were humanely killed at 132 ± 2d gestation and fetuses were delivered by hysterotomy. Fetal and heart weights were recorded. Isolated cardiomyocytes were stained with Ki67, a marker for proliferation. Isolated cardiomyocytes were also stained with myosin to measure cardiomyocyte size.

Leu^{27}IGF-2 infused into the left circumflex coronary artery did not increase fetal carotid mean arterial blood pressure and heart rate. Infusion of Leu^{27}IGF-2 leading to an increased IGF-2R signalling did not increase fetal weight, heart weight or left ventricle cardiomyocyte proliferation; however, it increases left ventricle cardiomyocyte width and area.

In summary, our results show that in fetal life, the IGF-2R signalling pathway does not mediate cardiomyocyte proliferation but does stimulate cardiomyocyte hypertrophy in fetus.

PIII-275

Sex Differences in the Association between Fetal Growth and Childhood Hearing Level: The Newcastle Thousand Families Study. Mark S. Pearce[1], Kay D. Mann[1], Raphael Nedellec[1,2], Adrian Rees[3]. *[1]Institute of Health and Society, Newcastle University, United Kingdom; [2]Ecole Nationale de la Statistique et de l'Analyse de l'Information, France; [3]Institute of Neuroscience, Newcastle University, United Kingdom.*

While current research priorities include investigations of age-related hearing loss, there are growing concerns regarding effects on childhood hearing. By utilizing historical data, it is possible to assess what factors may have increased hearing problems in children in the past (in an era without damage through (e.g., personal headphones), and this may be used to inform current public health policies to protect children against hearing loss and in turn reduce the long-term burden on individuals and services that may possible evolve. The aim this study was to investigate which factors in early life significantly impacted on hearing level in childhood using existing data from the Newcastle Thousand Families Study.

All 1142 children born in Newcastle in May and June 1947 to mothers resident in the city were initially recruited. Hearing function, through audiograms at 11 frequencies between 125Hz and 12kHz, was assessed at age 14 years in 174 study members. The data for frequencies 500Hz and 1000Hz were analysed in relation to detailed, prospectively recorded information on the birth, growth, development and social circumstances throughout childhood using linear regression on weighted means between the better and worse ear using the ratio 4:1 for each frequency.

Complete data were available for 147 individuals (64 female, 83 male). Birth weight (standardized for sex and gestational age) was significantly and positively associated with hearing level ($p \leq 0.001$), although on further sex-specific analysis this was limited to females. There was a significant interaction between sex and standardized birth weight on hearing level at both frequencies ($p \leq 0.001$). Other factors significantly, and independently, associated with hearing level were sex (better hearing in boys, $p<0.02$) and total number of prior ear infections ($p<0.03$). There were no associations with raw birth weight.

These results confirm the hypothesis that early growth is associated with hearing in childhood, although it appears to differ between males and females and is in the opposite direction to that expected. This finding is independent of early ear infections and suggests the need for both low and high rates of fetal growth to be assessed when determining early origins of later health.

PIII-276

Inflammatory Markers and Cardiovascular Risk: The Australian Aboriginal Birth Cohort Study 1987-2011. Gurmeet R. Singh, Susan M. Sayers. *Division of Child Health, Menzies School of Health Research, Charles Darwin University, Australia.*

Inflammatory processes are now recognized to play a central role in the pathogenesis of atherosclerosis. C- reactive protein (CRP), an acute phase reactant, has a direct role in atherogenesis. The angiopoietins 1 & 2 regulate angiogenesis. Angiopoietin-2 (Ang2) has a complex physiological role; local conditions influence whether Ang2 promotes or blunts tyrosine kinase receptors in endothelial cells. High levels of Ang2 have been found in plaques.

AIM: to examine the relationships of selected inflammatory markers (CRP and Ang2) to cardiovascular risk factors.

STUDY DESIGN: Cross-sectional snapshot in a prospective longitudinal cohort study in the Northern Territory

SUBJECTS: Young Aboriginal adults between 16-20 years of age; wave 3 of the cohort study

OUTCOME MEASURES: Inflammatory markers (CRP and Ang2), birth characteristics (birth weight, birth length and gestational age), current size (weight, height, BMI, waist circumference), BP, Lipids, HBA1c and residence (surrogate measure of socioeconomic status).

Of the original 686 recruited, 469 participants were seen in w3; 440 had available CRP and Ang2. Mean age was 18.2 years (range 16-20.2); 49.4.2% were males. CRP was significantly correlated with BMI >25kg/m^2 {3.2 [2.3,4.4] p=0.000}, higher waist circumference (>88 cm in females,>102 cm in males) {3.3 [2.5,4.4] p=0.000}, higher HbA1c (> 6.1%) {3.2 [1.03,10.2] p=0.045} and lower HDL-cholesterol (<1.0mml/L) {1.6 [1.2,2.1] p=0.000}. Ang2 was significantly higher in females {1.2 [1.1,1.4] p=0.000}, remote

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dwellers (1.2 [1.04,1.4] p=0.014} and those with systolic BP>120 mmHg {1.2 [1.1,1.4] p=0.00}. There were no correlations with birth weight or gestational age.

At this young age, rates of abnormal chronic disease risk markers are low. Inflammatory markers are showing correlations with higher, but not yet abnormal, levels of BMI, waist, HDL-cholesterol and systolic BP. Maybe this represents the early harbingers of chronic disease in this population, which has increasing obesity and high rates of cardiovascular disease in middle-age.

PIII-277

Body Composition in Adult Survivors of Severe Childhood Malnutrition.

Suzanne Soares-Wynter[1], Alan T. Barnett[1,2], Debbie S. Thompson[1], Michael S. Boyne[1], Clive Osmond[1,3], Mark A. Hanson[4], Peter D. Gluckman[5], Terrence E. Forrester[1]. *[1]Tropical Medicine Research Institute, The University of the West Indies, Mona, Jamaica; [2]Department of Surgery, Radiology, Anaesthesia and Intensive Care, The University of the West Indies, Mona, Jamaica; [3]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom; [4]DOHAD Division, School of Medicine, University of Southampton, United Kingdom; [5]UK Centre for Human Evolution, Adaptation and Disease, Liggins Institute, University of Auckland, New Zealand.*

Children with severe childhood malnutrition (SCM) may be non-oedematous (marasmus) or oedematous (kwashiorkor and marasmic-kwashiorkor). These syndromic differences could be developmentally determined and persist into adulthood. We hypothesized that adult survivors of marasmus (SM) would have more fat mass and survivors of kwashiorkor (SK) would have more fat-free mass.

We recruited 241 adults (151 men) who had SCM within the past 50 years. Anthropometry and body composition (by DEXA)was measured. Birth weight was obtained from hospital records. Data were adjusted for age and sex.

There were 86 SM, 77 SK and 78 survivors of marasmic-kwashiorkor (SMK). Compared to SM, SK were taller (168.9 vs. 166.3 cm; p=0.02), heavier (68.9 vs. 60.0 kg; p<0.001) and had greater BMI (24.2 vs. 21.6 kg/m2; p<0.001), fat-free mass (50.5 vs. 46.2 kg; p<0.001) and bone mass (3.15 vs. 2.79 kg; p<0.001). They had greater fat mass (15.2 vs. 10.7 kg; p=0.001), % fat (21.9 vs. 17.8%; p=0.003), and waist circumference (80.5 vs. 74.8 cm; p<0.01). SMK had intermediate values. After adjusting for height, these comparisons remained significant. After adjusting for BMI, only differences for fat-free mass and bone mass were significant.

Mean birth weight of SK was higher than that of SM (3.06 vs. 2.60 kg; p<0.001), while SMK was intermediate (2.85 kg). Adjusting for birth weight scarcely changed the body composition differences.

Multiple regression analyses including stunting, wasting, oedema and birth weight were performed. Oedema was associated with fat-free mass, fat mass and %fat. Birth weight was positively associated with fat-free mass, but only in SM (P for interaction= 0.03). It was not associated with fat mass or %fat.

SK had more fat-free mass, however, SM had less fat mass. Children with lower birth weights do not tend to develop oedema when exposed to malnutrition, unlike children who are heavier at birth. Birth weight was a predictor of fat-free mass but only in adult SM.

PIII-278

Comparing Insulin Sensitivity in Adult Survivors of Malnutrition.

Debbie S. Thompson[1], Michael S. Boyne[1], Clive Osmond[1,2], Christopher D. Byrne[2], Alan T. Barnett[1,3], Mark A. Hanson[4], Peter D. Gluckman[5], Terrence E. Forrester[1]. *[1]Tropical Medicine Research Institute, The University of the West Indies, Jamaica; [2]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom; [3]Department of Surgery, Radiology, Anaesthesia and Intensive Care, The University of the West Indies, Jamaica; [4]DOHaD Division, School of Medicine, University of Southampton, United Kingdom; [5]Centre for Human Evolution, Adaptation and Disease, Liggins Institute, University of Auckland, New Zealand.*

The polar extremes of severe childhood malnutrition, kwashiorkor (the oedematous form) and marasmus (the non-oedematous form) may have different developmental origins. We hypothesized that there could be a difference in insulin sensitivity between adult survivors of marasmus (SM) and kwashiorkor (SK).

We recruited 20 SM (mean age 24.9 ± 5.5 years, mean BMI 22.7 ± 4.4 kg/m2) and 20 SK (age 29.1 ± 8.9 years, BMI 24.3 ± 2.0 kg/m2). Anthropometry, DEXA scanning, a 150-minute hyperglycaemic euglycaemic clamp and a 120-minute oral glucose tolerance test were undertaken to assess body composition, insulin sensitivity, glucose tolerance and β-cell function. Whole body insulin-mediated glucose uptake (M-value; mg/kg/min), M/I (M-value/mean insulin concentration), glucose tolerance (at 0 mins and 120 mins) and ins30-0/glu30-0 (an index of β-cell function) were calculated. Age and sex adjusted multivariate analyses were used to compare the mean differences in anthropometry, body composition, insulin sensitivity, β-cell function and glucose tolerance between SM and SK.

There were no differences between the groups with respect to current weight, BMI, fat free mass and fat mass. Comparing survivors of marasmus and kwashiorkor, there were no differences (geometric mean ± geometric SD) in M- value (8.24 ± 1.44 vs. 7.89 ± 1.67; p=0.8), M/I (5.08 ± 2.16 vs. 4.97 ± 1.99; p=0.9) and ins30-0/glu30-0 (20.60 ± 1.86 vs. 21.84 ± 2.15; p=0.8). Three SM and 4 SK were found to have glucose intolerance. The areas under the curve during the OGTT for glucose and insulin showed no differences between the groups (P-values ≥ 0.7).

These data suggest that there is no difference in insulin sensitivity, glucose tolerance or β-cell function among non-obese adult survivors of marasmus and kwashiorkor. It remains to be seen if these findings are also true in obese survivors of marasmus and kwashiorkor.

PIII-279

Parental Age, Assisted Reproduction, and Developmental Trajectory of Language during Infancy: The Hamamatsu Birth Cohort (HBC) Study.

Kenji J. Tsuchiya[1], Kaori Matsumoto[1], Hideo Matsuzaki[1], Yuki Nakamura[2], Hiroaki Itoh[2], Nori Takei[1,3]. *[1]Research Centre for Child Mental Developement, Hamamatsu University School of Medicine, Hamamatsu, Japan; [2]Department of Gynaecology, Hamamatsu University School of Medicine, Hamamatsu, Japan; [3]Institute of Psychiatry, King's College London, United Kingdom.*

Advanced paternal age at birth may be associated with delayed language development and other types of aberrant neurocognitive development (Hvidtjørn *et al.*, 2009; Saha *et al.*, 2009; Hultman *et al.*, 2010). Assisted reproductive techniques (ART) can be a possible account for this association (Zhu *et al.*, 2009; Hvidtjørn *et al.*, 2010), since use of ART is usually accompanied by delayed conception and thus by advanced age of the couples (Ford *et al.*, 2000).

We aim at examining whether advanced paternal age is associated with delayed language development, and whether the association, if any, is accounted for by the use of ART.

The participants are 14-month-old infants and the mother, who take part in the Hamamatsu Birth Cohort (HBC) Study and had been longitudinally followed from foetal period. Language development of the participating infants was assessed at the 1st, 4th, 6th, 10th and 14th months using Receptive Language Score and Expressive Language Score of the Mullen Scales of Early Learning (Mullen, 1995). Demographic variables including parental age were also collected. Pregnancy and obstetric information was collected from the participating mothers and medical records during their pregnancy.

455 mother and infant dyads participated in this study. Mean scores of receptive language at 14th month were significantly lower in infants with father's age at birth ≥35 years and 25-34 years compared to infants with paternal age at birth <25 years. This association was not accounted for by other demographic factors. ART partly accounted for this association, although the association between advanced paternal age at birth and lower score in receptive language remained significant after controlling for ART in the multivariate analysis.

Paternal age at birth increase risk for delay in language development. This association was accounted for partly by use of assisted reproduction.

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PIII-280

Toxic Matters: The Need for Improved Policy To Prevent Developmental Exposure to Environmental Contaminants. Patrice Sutton, Tracey J. Woodruff. *Program on Reproductive Health and the Environment, University of California, San Francisco, CA, USA.*

All pregnant women in the U.S. have measured levels of multiple environmental chemicals linked to cancer or other adverse health outcomes in adulthood. To identify opportunities for preventing developmental exposure to toxic chemicals, we analyzed the regulatory framework that governs the presence of these chemicals in commerce.

Summarized U.S. data on: (1) population exposure to toxic chemicals in the environment; (2) policy that governs the presence of toxic chemicals in commerce; and (3) current efforts to foster policies with the capacity to prevent exposure to toxic chemicals.

In the U.S., developmental exposure to chemicals with reproductive and/or other toxicities is ubiquitous. Exposures are generally not controllable at the individual level (e.g., air and water pollution), and thus preventing exposure requires society wide policy action. The Toxic Substances Control Act of 1976 (TSCA) provides the U.S. Environmental Protection Agency (USEPA) with authority to take regulatory action on hazardous chemical substances. Presently TSCA allows minimal to no testing for industrial chemicals prior to entry into or to be on the marketplace. The vast majority of the approximately 83,000 chemicals in U.S. commerce today have entered the marketplace without comprehensive and standardized information on their reproductive or other chronic toxicities. At least five government studies conducted between 1984 and 2005 concluded that TSCA has not served as an effective vehicle for the public, industry, or government to assess the hazards of chemicals in commerce or control those of greatest concern. In 2009, the USEPA established "Essential Principles for Reform of Chemicals Management Legislation" to help inform legislative efforts now underway to reauthorize and significantly strengthen the effectiveness of TSCA. The lack of evidence of a chemical's safety prior to widespread population exposure is also recognized to be a policy gap by the American Medical Association, and broad coalitions of non-governmental organizations and industry.

An improved regulatory framework for chemicals in commerce is fundamental to preventing developmental exposures to toxic chemicals. Thus, TSCA reform is a key point of policy intervention for health professionals and researchers.

PIII-281

Programming of the Adrenal Function and Leptin Production by Nicotine Exposure during Lactation: Gender Differences in Rats. Isis Hara Trevenzoli[1], Egberto Gaspar Moura[2], Elaine Oliveira[2], Magna Cottini da Fonseca Passos[2], Cintia Rodrigues Pinheiro[2], Ana Paula Santos-Silva[2], Alex Manhães[2], Viviane Younes-Rapozo[2], Sylvio Claudio-Neto[2], Patricia Cristina Lisboa[2]. *[1]Laboratory of Molecular Endocrinology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [2]Laboratory of Endocrine Physiology, State University of Rio de Janeiro, Rio de Janeiro, Brazil.*

Neonate male rats whose mothers were nicotine-treated during lactation have higher adiposity, hyperleptinemia, hypercorticosteronemia, higher adrenal catecholamines, lower tyrosine hydroxylase (TH) and hypothyroidism. At adulthood, they present obesity, hyperleptinemia, leptin and insulin resistance and hypothyroidism. Here, we evaluated the adrenal function and leptin content in adipocytes and muscle of male and female adult offspring whose mothers were nicotine-treated during lactation.

On the 2nd postnatal day, dams were subcutaneously implanted with osmotic minipumps releasing nicotine (NIC - 6mg/Kg/day) or saline for 14 days. Male and female offspring were killed when they reached 180 days-old (1 rat from each litter).

Male NIC offspring presented higher adrenal catecholamine content (+89%) and TH expression (+38%), lower "in vitro" catecholamine release (-19%) and higher adrenergic β3 receptor (ADRB3, +59%) content in visceral adipose tissue (VAT). This group had hypercorticosteronemia (+77%), coherent with the increase of both CRH and ACTH immunostaining in hypothalamus and pituitary, respectively. Leptin content was higher in VAT (+23%), which may justify its hyperleptinemia. Female NIC offspring presented lower ADRB3 content in VAT (-39%) and lower leptin content in subcutaneous adipose tissue (-46%) but higher leptin content in soleus muscle (+22%), although leptinemia was normal.

We evidenced a sex dimorphism in the model of programming by maternal nicotine exposure during lactation. The adrenal function in adult offspring was programmed only in male offspring while the female offspring displayed relevant alterations in the adipose tissue and leptin content.

PIII-282

Influences of Gestational and Postnatal Exposure to Air Pollution and Hypercholesterolemic Diet on Atherosclerosis Progression. Mariana Veras[1], Felipe Fittipaldi[2], Maria Peres[1], Elia Caldini[1], Nilsa Damaceno-Rodrigues[1], Marcelo Zugaib[2], Rossana Francisco[2], Dulcinéia Abdalla[3], Paulo Saldiva[1]. *[1]Medicine School, USP, Brazil; [2]Hospital das Clínicas, Brazil; [3]Pharmacy School, USP, Brazil.*

It has long been known that maternal hypercholesterolemia during pregnancy is associated with accelerated postnatal progression of atherosclerosis (AT). Animal and human studies have also shown that exposure to air pollutants accelerate the development of atherosclerotic plaques and calcification in adults. In this study we investigated whether gestational and/or postnatal exposure to air pollution coupled with a high fat diet in adult life could also accelerate aortic plaque formation.

To test this, LDLr-/- mice were exposed during pregnancy (0.5-18.5 dpc) to either filter or polluted air, group F and P respectively, using a Particle Concentrator (daily dose=600 µg/m³ of PM2.5). After weaning period, pups were subdivided and new four groups formed, according to gestational and continuous or not postnatal exposure to air pollution, groups FF, FP, PF, PP (the first and the second letters refer to gestational and postnatal exposure to air pollution). Reaching the age of four months these groups were again subdivided and a hypercholesterolemic diet (D) introduced and a total of eight groups were formed, FF, FFD, FP, FPD, PF, PFD, PP and PPD. After 16 weeks of diet we assessed the progression of atherosclerotic plaque formation by ultrasound biomicroscopy.We measured plaque area (PA) in the vascular region proximal to the minor side of the aortic arch.

Mean PA (SD) in FF, FP, PF and PP was 0.01(0.01), 0.08(0.01), 0.13(0.01), 0.25(0.07) mm² respectively. In groups fed a high fat diet mean PA (SD) in FFD, FPD, PFD and PPD was 0.9(0.2), 1.08(0.4), 1.1(0.2), 1.4(0.4) mm² respectively. Results show that diet is the major factor involved in plaque progression ($p<0.001$). When groups fed normal diet are analyzed separately we observed that gestational exposure is associated with increased PA ($p=0.03$) and there is an interaction effect ($p<0.001$) between pre and postnatal exposure to air pollution and the size of the plaque.

Our preliminary results indicate that there is a possible influence of prenatal exposure to particulate air pollution and the AT progression in adult life. Although different treatments in high fat diet group did not attain significance, we could not reject the hypothesis that pre and postnatal exposure influence plaque development.

PIII-283

Longitudinal Growth Curves and Adolescent Height in Children with ADHD: Effects of Maternal Smoking during Pregnancy and a Decade of Treatment with Stimulant Medication. Tim Wigal, Annamarie Stehli, James Swanson. *Pediatrics, UC Irvine, CA, USA.*

We addressed two factors known to suppress growth and childhood height using data from the Multimodal Treatment Study of Children with ADHD (MTA), a 14-month randomized clinical trial (RCT) of 579 cases (children with ADHD who were 7-9 years of age at baseline) contrasting well-established treatment modalities, medication management (Med) and behavior modification (Beh), with observational follow-up assessments at 2, 3, 6, 8, and 10 years after baseline.

We applied modern auxological methods to describe three phases of growth: the childhood phase characterized by minimum height velocity (MHV) between 7-9 yrs in females and 9-11 yrs in males, the adolescent phase characterized by peak height velocity (PHV) between 9-11 yrs in females and 11-13 yrs in males, and predicted adult height attained after gradual decline in height velocity to zero usually by 20 yrs. The AUXAL Bayesian solution for the JPA2 model converged for 542 cases (435 males and 107 females) and generated eight milestones of human growth (age, height, and height velocity at MHV and PHV; adolescent gain; predicted adult height). Parent interview provided information on exposure to maternal smoking during pregnancy (Yes or No) and administered at each assessment provide information on pattern of exposure to stimulant medication (Never/Rarely,

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Sometimes, Always/Usually) and total exposure (mg in methylphenidate equivalents). ANOVAs were performed to evaluate effects of exposure to Maternal Smoking and Stimulant Medication on AUXAL milestones.
In males (n = 435) the ANOVA main effects were statistically significant (.01) for height in childhood (at MHV), adolescence (at PHV), and in adulthood, with an overall height reduction of about 2 cm for exposure to Maternal Smoking and about 0.3 cm/gram for exposure to Stimulant Medication. The interaction as significant for adult height, with the maximum effect of Stimulant Medication at the low doses with exposure to Maternal Smoking but at the high doses without exposure to Maternal Smoking. Effect size was larger for females (n = 107) than males.
The interaction of these two risk factors for height suppression suggests a threshold effect (maximum at a low dose exposure in the presence of exposure to Maternal Smoking and at a high dose in the absence of exposure to Maternal Smoking), with an asymptote for height suppression in the MTA of about 2.5 cm in males and 6.0 cm in females.

PIII-284

Contamination of Drinking Water in a Coal Mining Region: An Integrated Approach to the Assessment of Birth Defect Risk. Xiaoying Zheng[1], Guiying Cao[2], Bingzi Zhang[1], Tatiana Ermolieva[2], Jilei Wu[1], Ian Mccallum[2], Lijun Pei[1], Xinmin Song[1]. *[1]Peking University, Institute of Population Research, Beijing, China; [2]International Institute for Applied System Analysis, Austria.*
Drinking water quality is a serious problem in rural China.This study is to assess the relationship between level of drinking water contamination and human reproductive health in Shanxi province, a coal- mining region with one of the highest rates of birth defects in the world.
The study combines GIS-based modeling and structural equation methodologies.
Results show that mining critically influences the concentration of trace elements in drinking water and the consequent presentation of birth defects.
As China's rural areas have transformed from primarily agriculture-based economies to agro-industrial modes, corresponding policies and practice for minimizing environmental and health risks have lagged behind. In particular, the assured provision of clean drinking water should be a high priority for local development in the emerging economy.

PIII-285

Contents of Heavy Metals in Arable Soils and Birth Defect Risks in Shanxi, China: A Small-Area Level Geographical Study. Xiaoying Zheng, Lihua Pang, Jilei Wu, Lijun Pei, Linfang Tan, Cun Yan, Xinmin Song.
The burgeoning demands of China's urbanization and industrial development put pressure on the resources of the entire country, and have direct and indirect effects on the health of individuals, at times in areas far removed from cities themselves. Current evidence suggests that heavy metal pollution in soil, a common by-product of coal mining and other industrial activities, may be linked to risk of birth defects. We examine this hypothesis using small area level data including soil samples and detailed birth records from 2002–2004 from 120 villages in Shanxi province, a heavy coal mining region.
We examine this hypothesis using small area level data including soil samples and detailed birth records from 2002–2004 from 120 villages in Shanxi province, a heavy coal mining region.
We find that soils containing arsenic, lead, and nickel are significantly correlated with the incidence of birth defects. In particular, we find a strong positive dose-dependent association of birth defects with lead, a moderate positive effect with arsenic, and a dose-dependent negative association with nickel.
These results are consistent with the postulated link between arsenic and lead and human birth defects, but raise questions about the effects of nickel in this context. China's rapid urbanization underscores the need for closer attention to the relationship between health and the environment.

PIII-286

Early Life Programming of Age of Menarche Is Not Modified by Childhood Lead Exposure in Urban South African Females: Birth to Twenty Cohort. Shane A. Norris[1], John M. Pettifor[1], Linda Richter[1], David Dunger[2]. *[1]MRC/WITS Developmental Pathways for Health Research Unit, Department of Paediatrics, University of the Witwatersrand, Johannesburg, South Africa, South Africa; [2]Paediatrics, University of Cambridge, United Kingdom.*
We have shown that greater lead exposure in childhood delays the onset of menarche in black South African females. The aim of the study was two-fold: firstly, to investigate the association of birth weight and conditional weight gain in infancy and mid-childhood with age of menarche in urban, black South African females, and secondly, to determine the extent these associations are modified by environmental lead exposure.
The Birth to Twenty (Bt20) is a prospective birth cohort (n=3273) defined by the timing of a singleton birth within a specified period (late April to early June) in 1990, as well as continued residence within the metropolitan area of Johannesburg-Soweto, South Africa for at least six months after the birth of the child. We defined early life exposures as birth weight at delivery, and measured weight at age two and five years. Using longitudinal data collected annually from age nine to 16 years, onset of menarche in females was determined. At age 13 years, blood lead levels were measured in the cohort as a marker of environmental exposure to lead. Conditional weights (z-scores) were derived for infancy (birth to two years) and mid-childhood (two to five years). Multiple regressions were used to examine the association between birth weight, conditional weights, age of menarche and lead exposure.
The mean age (SD) of menarche in the analytical sample of black females (n=907) was 12.7 (1.2) years. Birth weight and infant conditional weight was not significantly associated with age of menarche. However, mid-childhood conditional weight was significantly negatively associated with age of menarche (-0.31; p=0.006). As previous shown, lead exposure was significantly correlated with age of menarche (pearson correlation=0.16; p<0.001), but did not modify the association between mid-childhood confitional weight and age of menarche.
Independent of later childhood environmental exposures known to influence onset of menarche, for every SD greater weight gain between age two and five years results in a reduction of four months in the age of menarche. This data highlights a developmental period that may have contributed to the secular trend of decreasing age of menarcher in South African black females.

PIII-287

Maternal Protein Restriction (MPR) in Pregnancy and/or Lactation Affects Seminiferous Tubule Organization in Male Rat Offspring (OFF). Guadalupe L. Rodriguez[1], Rosa M. Vigueras-Villasenor[2], Raquel Trejo[3], Nadia E. Moran[1], Fernando Larrea[1], Peter W. Nathanielsz[4], Elena Zambrano[1]. *[1]Reproductive Biology, INCMNSZ, Mexico City, Mexico; [2]INP, Mexico City, Mexico; [3]UAM, Mexico City, Mexico; [4]Center for Pregnancy and Newborn Research, Dept OB/GYN, The University of Texas Health Science Center San Antonio, San Antonio, USA.*
Sertoli cells (SC) play a central role in the control of germ cell (GC) proliferation and differentiation from fetal to adult developmental stages. Normal spermatogenesis depends on specific seminiferous tubule architecture and continuous cross-talk between SC and GC. SC functioning is dependent on the number and type of GC associations. Based on previous results showing that MPR during pregnancy impaired reproduction of male OFF (J Phys 563:275), we investigated whether MPR has deleterious effects on SC function as well as organization of GC during the first wave of spermatogenesis.
We studied male OFF of rats fed control (C) (20% casein) or a restricted (R) (10% casein) isocaloric diet in pregnancy (first letter) and/or lactation (second letter) of four groups CC, RR, CR or RC. At postnatal day (PND) 21 and 36, male OFF were sacrificed and testis were removed and fixed to evaluate androgen receptor (AR), tubular lumen formation and seminiferous tubule organization. Data MSEM; analysis by ANOVA; n=5.
At PND 21 and 36 more tubules had a clear lumen in CC vs. R groups. At 21 PND, the germinal epithelium (GEp) of CC group was developed until pachytene stage, which at this age is the maximum development attained;

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whereas the GEp of R groups was developed until preleptotene and leptotene stage. At 36 PND, the GEp of CC animals reached the cap and early acrosome phase of spermiogenesis. In contrast, Golgi phase of spermiogenesis was the latest stage in the GEp of the MPR groups. The expression of AR protein was similar in all groups, however, the cytoplasm projections of SC showed a stronger signal in CC animals than in R groups.
The results presented above suggested that MPR during pregnancy and/or lactation delay SC maturation, GC differentiation and affects intratubular organization.

PIII-288

Maternal High-Fat Feeding during Early Development Results in the Reduction of Ovarian Follicle Numbers in Adult Offspring in Mice. Khaled H. Sadek[1,2], Kimberley D. Bruce[1], Nick S. Macklon[1,2,3], Felino R. Cagampang[1], Ying C. Cheong[1,2,3]. *[1]Institute of Developmental Sciences, Academic Unit of Human Development & Health, Faculty of Medicine, University of Southampton, Southampton, United Kingdom; [2]Obstetrics and Gynaecology, Princess Anne Hospital, Southampton University Hospital NHS Trust, Southampton, United Kingdom; [3]Complete Fertility Centre Southampton, Princess Anne Hospital, Southampton University NHS Trusts, Southampton, United Kingdom.*
Maternal nutrition and body composition during pregnancy can have long-term consequences on the future health of the offspring. Exposure of the developing fetus to poor maternal nutritional environment leads to increased risk of cardiometabolic diseases in adulthood. Nevertheless, the effects of maternal nutrition during fetal development on future reproductive health of the adult offspring remain to be elucidated. Obesity is associated with subfertility which in women is linked to ovarian dysfunction. The objective of this study was to investigate whether feeding a high fat(HF) diet to pregnant mice alters the ovarian morphology in the adult offspring.
Female C57/BL6 mice were fed either a HF diet (45% kcal fat) or a standard chow diet (C; 21% kcal fat) from four weeks prior to conception, through pregnancy and lactation. At 15 weeks of age (equivalent to early adulthood in humans), female offspring were killed and ovaries were dissected and processed for immunohistochemistry. Ovarian sections in offspring from HF-fed (n=22) and C-fed dams (n=27) were stained by immunohistochemistry with an anti-mullerian hormone antibody to assess the number of AMH-positive follicles. Stereological counting of follicle numbers were done blind on seven sections (50μm apart) per animal. In addition, representative sections were histologically stained with hematoxylin and eosin. Non-parametric data was analysed using Mann-Whitney U test, and values are expressed as mean±SEM.
There was a 1.4 fold reduction in total number of antral and graafian follicles in ovaries of adult offspring from HF-fed dams vs. offspring from C-fed dams ($p=0.004$). This reduction was accompanied by a 1.6 fold lesser number of graafian follicles in HF offspring vs. C offspring ($p=0.023$). The number of antral follicles were similar in both offspring groups.
These morphological changes in the offspring ovaries suggest that maternal HF-feeding during pregnancy may impact on gonadal development and function, which in turn may contribute to increased subfertility in adulthood.

PIII-289

Early Life Exposure to a Bacterial Mimetic Impairs Reproductive Function in Rats. Luba Sominsky, Adam K. Walker, Deborah M. Hodgson. *School of Psychology, The University of Newcastle, NSW, Australia.*
Exposure to infection in early life is known to produce long-term neuroendocrine and immune alterations. Our previous findings indicated that administration of lipopolysaccharide (LPS) during the neonatal period results in increased anxiety-like behaviour in rats, associated with alterations in HPA axis activity, increased central cytokine levels and microglial activation. Given the close communication between the HPA and HPG axes, we investigated whether exposure to a bacterial mimetic may also lead to alterations in reproductive development.
Male and female Wistar rats were administered either LPS (0.05 mg/kg, ip) or non-pyrogenic saline on days three and five postpartum. The effect of neonatal treatment on HPA and HPG activity was assessed, as well as reproductive outcomes driven by these neuroendocrine systems; ie puberty onset and mating behaviour. The immediate and long-term effects of bacterial exposure on testicular development was also assessed.
Postnatal exposure to LPS induced an immediate increase in corticosterone levels and significantly altered puberty onset and reproductive behaviour in both sexes ($p<0.05$). Neonatal LPS suppressed gonadal hormones throughout puberty, during mating in adulthood, and into late adulthood. Data from males indicated altered gonocyte development, by reduced gonocyte genesis, immediately following LPS exposure and increased epithelial disorganization of the seminiferous tubules in adulthood as compared to saline-treated controls ($p<0.05$).
These findings demonstrate the long-term impact of exposure to a bacterial mimetic during the critical neonatal period on later life reproductive development. Given the common occurrence of neonatal infection in the human population, the current research indicates possible pathways through which predisposition to subfertility may arise. We are currently characterizing the long-term impact of bacterial exposure on ovarian morphology and functioning which will be presented.

PIII-290

Increased Fertility and Mortality among Women Who Had Been Prenatally Exposed to Famine: Support for the Disposable Soma Theory. Annet F.M. van Abeelen[1,2], Marjolein V.E. Veenendaal[1], Rebecca C. Painter[3], Susanne R. de Rooij[1], Marcel G.W. Dijkgraaf[1], Patrick M.M. Bossuyt[1], Sjoerd G. Elias[2], Diederick E. Grobbee[2], Cuno S.P.M. Uiterwaal[2], Tessa J. Roseboom[1,3]. *[1]Department of Clinical Epidemiology, Biostatistics, and Bioinformatics, Academic Medical Center, Amsterdam, Netherlands; [2]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, Netherlands; [3]Department of Obstetrics and Gynecology, Academic Medical Center, Amsterdam, Netherlands.*
The disposable soma theory proposes that the two traits fertility and body maintenance are mutually balanced. Increased investments in one of these traits are traded off for a reduction in investment in the other. Previously, we demonstrated an increased reproductive success in women but not men who were exposed to famine in early gestation. Here we examine the association between prenatal famine exposure and adult mortality.
We studied adult survival among 1,991 term singletons born around the time of the Dutch famine. We compared overall and cause specific adult mortality (between 18 and 64 years of age) among persons exposed to famine in late, mid, and early gestation to those unexposed to famine *in utero* using Cox proportional hazard models. Since the associations may differ in the two sexes, we performed sex-specific analyses.
206 people (10%) had died by the end of follow-up. Compared to unexposed women, women exposed to famine in early gestation had a significantly increased overall adult mortality (HR 1.8, 95% CI 1.0 to 3.3) and cancer mortality (HR 2.3, 95% CI 1.1 to 4.8), and a higher risk of cardiovascular mortality (HR 3.2, 95% CI 1.0 to 10.8). Among these women there was a higher breast cancer mortality risk (HR 4.2, 95% CI 0.9 to 19.0). No such effects were observed in men exposed to famine in early gestation compared to unexposed men (HR 0.4, 95% CI 0.2 to 1.1 for overall adult mortality; HR 0.9, 95% CI 0.3 to 3.1 for cardiovascular mortality; and HR 0.3, 95% CI 0.0 to 1.8 for cancer mortality).
The results of this study support the disposable soma theory, at least in women. Women who were exposed to famine in early gestation have an increased reproductive success and higher overall adult, cancer and cardiovascular mortality risk. Among men, no such effects were observed.

PIII-291

Effect of Early Life Growth Restriction on Renal Function in Adulthood in Male and Female Rat Offspring. Kyungjoon Lim[1], Paul Lombardo[2], Michal Schneider-Kolsky[2], Kate M. Denton[3], Mary Jane Black[1]. *[1]Anatomy & Developmental biology, Monash University, Victoria, Australia; [2]Medical Imaging and Radiation Sciences, Monash University, Victoria, Australia; [3]Physiology, Monash University, Victoria, Australia.*

It is now well established that there are sex differences in the early programming of the fetus. We hypothesized that due to differences in renal programming between males and females, renal function will be impaired in male growth-restricted offspring in adulthood but not in females. The aim was to compare renal function in adult male and female rat offspring that had been growth-restricted in early life.

Early life growth restriction was induced in Wistar-Kyoto rats by maternal protein restriction during pregnancy and lactation. At 32 weeks of age kidney structure and function were comprehensively assessed using M mode and Doppler ultrasound and 3H inulin and 14C para-aminohippurate clearance techniques.

Conscious mean arterial blood pressure and heart rate were unchanged in growth-restricted offspring. Overall, renal function was maintained with GFR/g kidney weight not different to the controls. Relative kidney length was significantly increased in growth-restricted offspring (9% increase in male and 7% increase in female IUGR kidneys when compared to controls) and there was evidence of alterations in renal function; of importance, there was a significant increase in filtration fraction (15% increase in male and 14% increase in female IUGR kidneys) indicative of glomerular hyperfiltration. Male and female offspring responded in a similar manner to IUGR, however, in general female offspring exhibited a higher level of renal function when compared to male offspring.

Although early life growth restriction led to altered renal function in adulthood there was no evidence of differential programming of impaired renal function between the sexes.

PIII-292

Uteroplacental Insufficiency Extends the Period of Nephrogenesis through the GDNF/cRet Pathway. Karen M. Moritz[1], Shannyn Rosser[1], Mary E. Wlodek[2]. *[1]The University of Queensland, Australia; [2]The University of Melbourne, Australia.*

Intrauterine growth restriction increases the risk of hypertension in adulthood. We have shown that growth restriction caused by uteroplacental insufficiency in the rat is associated with a nephron deficit in offspring. Our aim was to determine if the reduced nephron endowment was due to impaired branching morphogenesis and/or early cessation of nephrogenesis.

Uteroplacental insufficiency was achieved by bilateral uterine vessel ligation (Restricted, R) or Sham (Control, C) surgery on WKY rats on pregnancy day 18. Kidneys were collected for gene and protein studies (real-time PCR, Western blot) at day 20 of gestation and for gene expression and histology at postnatal day 7 (PN7), an age when nephrogenesis is nearing completion in the rat.

Uteroplacental insufficiency resulted in growth restriction with R offspring significantly smaller than C at birth and remaining so at day 7 (33% in males; 27% in females). At day 20, there was no difference in gene expression levels of glial cell line-derived neurotrophic factor (GDNF, a major regulator of branching morphogenesis) but its major receptor, cRET was elevated ($P<0.05$) in kidneys of Restricted fetuses. At PN7 both GDNF and cRET were higher in R offspring compared to C ($P<0.01$ for treatment). Western blot identified a strong tendency for increased GDNF protein at PN7 ($P=0.06$) in R offspring. At PN7, histological studies demonstrated there was still a prominent nephrogenic zone present in the kidneys of R offspring with obvious immature glomeruli. However, in the C kidneys, nephrogenesis appeared complete with no evidence of a nephrogenic zone in any sections examined.

Uteroplacental insufficiency extended the period of nephrogenesis which was associated with an upregulation of GDNF and cRET suggesting branching morphogenesis was ongoing. We conclude the reduced nephron endowment in R offspring probably occurred earlier in development. Further investigation of factors regulating kidney development during fetal and early postnatal life is required to elucidate the mechanisms involved. We suggest the extension of nephrogenesis represents an attempt by the kidney to restore nephron number although this is insufficient to restore nephron number to that of C offspring.

PIII-293

Fetal Programming of Erythropoietin Gene Expression in the Rat Kidney. Kai D. Nüsken[1], Eva Nüsken[1], Yvonne Birkner[2], Holm Schneider[2], Jörg Dötsch[1]. *[1]Department of Pediatrics, University of Cologne, Germany; [2]Department of Pediatrics, University of Erlangen-Nuremberg, Germany.*

Intrauterine growth restriction (IUGR) is associated with an increased erythropoietin (EPO) concentration in cord blood of human neonates. Experimental utero-placental insufficiency by bilateral uterine artery ligation induces both IUGR and an increased EPO gene expression in the neonatal rat liver, which is the primary site of EPO production at birth. This study aimed at clarifying whether there is also a long-term effect on renal EPO gene expression.

We studied offspring of dams treated by uterine artery ligation (LIG; n=5) or sham operation (SOP; n=12) compared with untreated controls (C; n=6). At an age of 30 weeks, retro-orbital blood samples were obtained and the hematocrit was measured. Furthermore, both kidneys were harvested and divided into cortex and medulla. In the renal cortex, expression of the EPO gene was quantified by RT-PCR.

EPO gene expression in renal cortex was increased in group LIG compared with group C (82 ± 22 vs. 46 ± 14 relative units, $p < 0.05$). Likewise, LIG animals showed a significantly higher hematocrit than group C ($70.5 \pm 1.6\%$ vs. $66.3 \pm 1.1\%$, $p < 0.01$).

Utero-placental insufficiency induces an elevation of renal EPO gene expression in later life. The elevation seems to be functionally and systemically relevant as shown by an increased hematocrit. A causal relationship to sequelae after IUGR is conceivable because of multiple "beneficial" (e.g. tissue-protective) as well as "adverse" (e.g. hypertensive) effects of EPO. Further studies are needed to clarify these issues.

PIII-294

Kidney Dysfunction in Adult Rat Offspring Programmed by Maternal Prolactin Inhibition during Lactation. Marco Aurélio Fonseca Passos[1], Magna Cottini Passos[2], Elaine Oliveira[2], José Firmino Nogueira-Neto[2], Isabela Teixeira Bonomo[2], Patricia Cristina Lisboa[2], Egberto Gaspar Moura[2]. *[1]Anatomy, State University of Rio de Janeiro, Rio de Janeiro, Brazil; [2]Physiological Sciences, State University of Rio de Janeiro, Rio de Janeiro, Brazil.*

Evaluate, during development, the renal function of rats whose mothers had hypoprolactinemia at the end of lactation.

Lactating Wistar rats were treated with bromocriptine (BRO, 1 mg twice a day, n=12) or saline (n=12) on days 19, 20 and 21 of lactation, and their offspring were followed from weaning until 180 days old. Body and kidney weights, sodium, potassium and creatinine were evaluated at two time points (90 and 180 days, one offspring from each litter). Values were considered significant when $p<0.05$.

Adult BRO-treated offspring presented higher body weight (10%), lower relative renal weight at 90 and 180 days (9.2% and 15.7%, respectively), glomerulosclerosis and peritubular fibrosis. At 90 and 180 days, creatinine clearance was lower (32% and 30%, respectively), whereas serum potassium was higher (19% and 29%, respectively) but there were no changes in serum sodium. At 180 days, we detected higher proteinuria (36%) and serum creatinine levels (20%).

Our data suggest that prolactin inhibition during late lactation programs renal function damage in adult offspring that develops gradually, first affecting the creatinine clearance and potassium serum levels with further development of hyperproteinuria and higher serum creatinine, without affecting sodium. Thus, precocious weaning can be a risk factor for further development of kidney disease.

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PIII-295

Effect of Maternal Low Protein Diet during Pregnancy and Lactation on the Expression of Renal Organic Anion Transporter-1 in the Rat Adult Offspring. Jacob Pearson[1,2], Barent DuBois[1,2], Anil D'Mello[3], Ganesh Cherala[1,2]. *[1]College of Pharmacy, Oregon State University, OR, USA; [2]College of Pharmacy, Oregon Health & Science University, OR, USA; [3]Department of Pharmaceutical Sciences, University of the Sciences in Philadelphia, PA, USA.*

To determine the effect of maternal low protein diet (LPD) administered during pregnancy and lactation on the status of renal Organic Anion Transporter 1 (OAT1) in the offspring.

Pregnant and lactating rats were fed either a purified control diet (19% protein, n=8) or a LPD (8% protein, n=7) throughout pregnancy and lactation and offspring were weaned onto rodent lab chow on postnatal day28. On day 65 and 150 post-birth, one male and one female offspring from each litter were sacrificed and kidneys were collected and snap frozen. Total Renal RNA was isolated using the TRIZOL method, cDNA was synthesized using iScript CDNA synthesis kit from Bio-Rad, and RT-PCR was carried out using Bio-Rad iQ SYBR Green supermix. Protein expression was quantified by western blotting using the following sub-cellular fractions: microsomes, cytosol, and plasma membranes.

A twofold decrease in the expression of renal OAT1 mRNA was observed in 65 day old male and female offspring in the LPD group. At day 150 there was a further decrease of 10-fold and 50-fold in OAT1 expression in the male and female LPD offspring, respectively. On the other hand, the protein expression in all sub-cellular fractions was unaltered in 65 day old male and female offspring in the LPD group. However, though statistically insignificant, a 2-fold higher protein expression was observed in 150 day old male and female offspring.

The mRNA expression of renal OAT1 exhibited long term decreases after exposure to perinatal low protein diet. However, the data on OAT1 protein expression is in discordance with mRNA expression suggesting possible alterations in post-transcriptional, translational, or post-translational processes. This study findings could explain the interindividual variability observed in the disposition of drugs interacting with OAT1.

PIII-296

Renal Function in Aging Male and Female Offspring Exposed to Maternal Malnutrition. Ryan J. Wood-Bradley, Sarah L. Henry, Roger G. Evans, Luise A. Cullen-McEwen, John F. Bertram, James C. Armitage. *Anatomy and Developmental Biology, Monash University, Victoria, Australia.*

Among other functions, the kidney plays a key role in blood pressure regulation, and renal development is programmed by the in utero environment. Previous studies indicate that folic acid (FA) administration prevents the development of increased blood pressure (BP) in offspring of protein-deprived rats but it is not known if supplementation with folic acid during development can affect renal function. We aimed to determine renal and cardiovascular function in one year old male and female offspring exposed to maternal protein restriction and FA supplementation/restriction.

Female Sprague-Dawley rats (n=5-8 per group) were fed a control (C, 18% casein, 2mg/kg FA), low protein (LP, 9% casein, 2mg/kg FA), low protein high FA (LP+F, 9% casein, 200mg/kg FA) or low protein low FA (LP-F, 9% casein, <0.05mg/kg FA) diet for three weeks prior to mating, and throughout pregnancy and lactation. After weaning, offspring were chow fed ad libitum. At one year of age glomerular filtration rate (GFR) and effective renal plasma flow (eRPF) were estimated in anaesthetised rats by measuring [3H]-inulin and [14C]-para-aminohippurate clearance. Mean arterial pressure (MAP) was determined by radiotelemetry. Data were analysed by two way ANOVA, weighted for litter with maternal diet and offspring sex as main factors.

MAP and GFR were similar in all groups. However, male and female offspring had differing eRPF depending on maternal diet exposure ($P<0.05$). Male offspring of control fed dams had lesser eRPF compared with those fed the experimental diets (eRPF/bw: C 7±3µl/min/g bw vs. LP 14±2µl/min/g bw, LP+F 11±2µl/min/g bw, LP-F 10±2µl/min/g bw, $p<0.05$). In contrast, female offspring of control fed dams had greater eRPF than those fed experimental diets (eRPF/bw: C 9±1µl/min/g bw vs. LP 5±2µl/min/g bw, LP-F 7±2µl/min/g bw $p<0.05$). Interestingly the LP+F females did not have reduced eRPF (12±3µl/min/g bw).

Maternal protein or FA intake in pregnancy does not programme elevated blood pressure in offspring at one year of age. However, exposure to maternal LP diet programmes sex specific alterations in renal blood flow. Males, but not females derived from a LP maternal diet exhibit hyperaemia. There is some evidence that high maternal FA intake may prevent reduction of eRPF in LP offspring but further studies are required.

PIII-297

Breastfeeding and Offspring Birth Weight in the Western Region of São Paulo: the Butantã Cohort. Ana Maria U. Escobar, Filumena Maria S. Gomes, Maria Helena Valente, Rafael R. de Moraes, Maria Teresa B. Fernandes, Alexandra Brentani, Isac de Castro, Sandra Josefina F.E. Grisi. *Pediatrics, Faculdade de Medicina da Universidade de São Paulo, SP, Brazil.*

Introduction: Exclusive breastfeeding (EBF) in the first months of life is important due to biological, psychological and sociocultural aspects. EBF protects against infant mortality and morbidity, with advantages for both mother and baby, especially babies with delayed intrauterine growth and low birth weight .Breastfeeding is a complex process which brings crucial benefits to the newborn, but requires a multidisciplinary orientation and supervision during pregnancy and the first days after childbirth, which is a critical period.

Objective: To determine if the newborn birth weight has influence on the time of exclusive breastfeeding.

A retrospective cross-sectional study comprising 1117 women and their children, followed in Butantã Cohort was conducted. Butantã cohort is located in the western region of São Paulo City and is part of a research project of the Pediatrics Department. Clinical history and data review from pediatric clinical records, including a collection of information on the duration of exclusive breastfeeding and birth weight were obtained. The cumulative frequency of weight was sequentially analyzed with Pearson's chi-square test for independent groups and expressed proportions. Odds ratio for risk estimate was calculated.

30% (n= 27) Low birth weight children (less than 2,500 kg) where never breastfed, compared to 6.8% (n=70) who breastfed ($p<0.0001$, OR = 5,859, 95% CI = 3511 to 9778). From the total sample, 12.9% (n=57) low birth weight children were breastfed less than three months, compared to 5.9 (n=40) children who breastfed at least three months. We found a significant difference ($p <0.0001$, OR = 2350 95% CI = 1539 to 3590) in breast feeding time comparing normal with low birth weight children.

there is a strong correlation between low birth weight infants and breastfeeding duration. Low birth weight children receiving breast feeding for a period under than three months or weren't breastfed at all. Future studies should be conducted in order to identify possible factors associated with early weaning of low birth weight infants.

PIII-298

Duration of Breastfeeding Is Associated with the Methylation of *LEP* in Young Children. Sylvia A. Obermann-Borst[1], P. Eline Slagboom[2], Paul H.C. Eilers[3], Elmar W. Tobi[2], Frank H. de Jong[4], Eric A.P. Steegers[1], Bas T. Heijmans[2], Régine P.M. Steegers-Theunissen[1,5]. *[1]Obstetrics and Gynecology, Erasmus MC, Netherlands; [2]Molecular Epidemiology, LUMC, Netherlands; [3]Biostatistics, Erasmus MC, Netherlands; [4]Endocrinology, Erasmus MC, Netherlands; [5]Epidemiology and Clinical Genetics, Erasmus MC, Netherlands.*

Epidemiological and experimental studies have shown that pre- and postnatal nutrition are involved in epigenetic effects on metabolic programming of the child and are associated with disease risk in later life. We hypothesize that breastfeeding influences the methylation of *LEP*, a non-imprinted gene implicated in appetite regulation and fat metabolism. We aim to investigate associations between: 1) birth weight as a proxy for prenatal nutrition state, gender and methylation of *LEP*; 2) breastfeeding, serum leptin concentration and the methylation of *LEP*.

Of 120 children (boys n=70) at 17 months of age genomic DNA was isolated from white blood cells and treated with bisulphite (Zymo Research). The methylation of seven CpG dinucleotides in the promoter region of *LEP*

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was measured by mass spectrometry (Epityper, Sequenom). Serum leptin concentration was measured in duplicate using a specific Human Leptin Radioimmunoassay kit (Millipore, St. Charles, MO). Breastfeeding was defined as any breastfeeding after birth, and as covariate with with scores ranging from 0-4 depending on the duration in months (none; <1, >1-3, >3-6, >6). Linear Mixed Model analysis was applied on the raw methylation data without imputation of missing values.

Boys had a higher birth weight (P=0.044) and showed lower *LEP* methylation (-7.3%; p=0.010) compared to girls. In the total group an inverse association was shown between *LEP* methylation (-5.0%, p=0.005) and birth weight per SD increase (+584g) which remained significant after adjustment for gender. Data on breastfeeding was available for 99 children, of which 74 were breastfed. Duration of breastfeeding was associated with a lower *LEP* methylation (–2.9%; p=0.010). Children who were breastfed for >1-3 months had a significant higher concentration of leptin (2.8 vs. 2.6 mmol/L; p=0.025).

Our data suggest that the child's gender, birth weight and breastfeeding are associated with epigenetic differences in *LEP*. Future studies must reveal if breastfeeding in early life and the associated decrease in *LEP* methylation is one of the epigenetic mechanisms by which the protective effect of breast feeding against childhood obesity is established.

PIII-299

Adipocyte Morphology and Leptin Signaling in Rat Offspring from Mothers Supplemented with Flaxseed during Lactation. Magna Cottini Passos[1], Patricia Cristina Lisboa[1], Mariana Sarto Figueiredo[1], Elaine Oliveira[1], Trevenzolli Hara Isis[2], Aline Andrade Troina[1], Celly Cristina Nascimento-Saba[1], Egberto Gaspar Moura[1]. *[1]Physiological Sciences, State University of Rio de Janeiro, Rio de Janeiro, Brazil; [2]Molecular Endocrinology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.*

We have recently shown that maternal flaxseed supplementation during lactation induces insulin resistance in the adult offspring. Here, we studied the effects of maternal dietary flaxseed during lactation on adipocyte morphology and leptin signaling in the hypothalamic-pituitary-thyroid axis in the adult progeny.

Lactating rats were divided in: controls (C, n=8) and flaxseed (F, n=8), with 25% of flaxseed. After weaning, pups received a standard diet until postnatal day (PN) 180. Male offspring (2 from each litter) were killed at PN21 and 180. All significant data were $p<0.05$.

Weaned F group presented lower total and subcutaneous fat mass, higher subcutaneous adipocyte area (+48%), but at adulthood they presented higher subcutaneous and visceral adipocyte areas (+40% and 1.9-fold increase, respectively), with no change in body fat mass. At PN21, F group had hyperleptinemia (+69%), lower T_3 (-33%), higher TSH (2.1-fold increase), higher pituitary leptin receptor (Ob-R, +11%), signal transducer and activator of transcription 3 (STAT3, +21%) and phosphorylated-STAT3 (p-STAT3, +77%) protein content. Adult F offspring only showed lower T_4 (-28%) and higher thyroid Ob-R (+52%) expression.

Thus, the maternal flaxseed supplementation decreases offspring adiposity and increases pituitary leptin signaling at weaning, but at adulthood, they showed hypertrophic adipocytes and higher thyroid leptin receptor. The present data constitutes a warning against extensive use of flaxseed during lactation.

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PIII-300

Fatty Acid Composition of Murine Milk Is Directly Affected by the Maternal Dietary Lipid Intake. Bert J.M. Van de Heijning[1], Diane Kegler[1], Annemarie Oosting[1], Eline M. Van der Beek[2]. *[1]Baby Nutrition, Danone Research-Centre for Specialised Nutrition, Netherlands; [2]Baby Nutrition, Danone Research-Centre for Specialised Nutrition, Singapore.*

Early life dietary lipids are important for a healthy growth and development. The fatty acid (FA) content of breast milk is mainly derived from body fat stores but partly also from dietary lipid intake during lactation. Previously we showed that animals raised on a diet enriched in n3 FA show lower adiposity when challenged with a Western style diet during adulthood.

To assess to what extent and how soon the dietary FA composition translates into milk, lactating mice (C57/BL6) were fed diets varying in FA composition. All diets were AIN-based, contained 10 w% lipids, and were provided to dams from day 2 after delivery onward (postnatal (PN) day 2). Three test diets were compared to a control diet: **n3 LCP**, a diet containing the n3 long chain polyunsaturated FA (LC-PUFA) eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids; **low n6**, a diet with linoleic acid (LA) levels reduced by 55%; and **MC**, a diet with more than twice the medium chain FA amount. In early, mid and late lactation (i.e. PN day 7-9, 10-12 and 13-15) milk samples were obtained from dams. Pup blood samples for erythrocyte membrane FA analyses were obtained upon weaning (PN day 21).

Irrespective of the maternal diet, milk content changed over time: saturated FA content (>50%) and medium chain FA increased, whereas monounsaturated FA content (<40%) and arachidonic acid (ARA) decreased. Milk PUFA levels remained stable between 10-15%.

Milk fat composition was already affected after five days of dietary intervention. The **n3 LCP** diet containing EPA and DHA induced a 6-7-fold increase of these n3 LC-PUFA in milk (which equals 30-40% of the amount in the diet). Milk ARA levels were reduced (-25%) due to the n3 diet. The **low n6** diet resulted in lower milk LA content. In contrast, milk medium-chain FA content was unaffected by the the **MC** diet.

The milk FA profile at late lactation (i.e. the pup's diet) was qualitatively reflected in the pup erythrocyte membrane similar as to how the dietary FA was translated into milk.

We conclude a direct and rather immediate effect of the maternal dietary lipid intake on the FA composition of breast milk, but the extent of changes in milk FA composition depends on the dietary FA profile supplied. Adaptations in the maternal diet, reflected in the milk FA composition, are effectively translated into the offspring's tissues.

PIII-301

Breastfeeding Attitudes and Practices among Chinese Mothers in Ireland: A Mixed Methods Study. Qianling Zhou, Katherine M. Younger, John M. Kearney. *School of Biological Sciences, Dublin Institute of Technology, Dublin, Ireland.*

Migration has potential influences on breastfeeding practices, and recent Irish studies have revealed a significant difference in breastfeeding initiation rate between nationals and non-nationals. This study aimed at filling an information gap in the breastfeeding attitudes and practices of the Chinese, one of the largest ethnic groups in Ireland, and exploring the factors in influencing maternal breastfeeding practices.

A sequential explanatory mixed methods approach was adopted. A cross-sectional self-administered retrospective survey (written in Chinese) was initially conducted among a convenience sample of Chinese mothers living in Ireland (n 322), recruited mainly in the Asian markets, using the snowball technique. Quantitative data were obtained from mailed questionnaires. To further explain the main quantitative findings, seven semi-structured focus groups were conducted among Chinese mothers who had given birth in Ireland (n 33). Qualitative data were analyzed by thematic content analyses.

Quantitative data demonstrated that while the breastfeeding initiation rate among Chinese mothers who gave birth in Ireland (CMI) (75.6%) was high and close to that of migrant Chinese mothers who gave birth in China (CMC) (87.2%), a significantly shorter duration of breastfeeding was found among CMI compared with CMC ($P<0.05$). Giving birth in Ireland was independently negatively associated with a longer duration of breastfeeding (≥ 4 months) (OR 0.22, 95% CI 0.08-0.56) among Chinese migrants. Qualitative results explained that this shorter breastfeeding duration was mainly due to insufficient family support, cultural conflicts, language barriers, and migrants' low socioeconomic status. Both quantitative and qualitative studies revealed a strong cultural belief in the efficacy of the traditional Chinese postpartum diet on milk production. Maternal consumption of such diet was independently positively associated with longer breastfeeding duration (OR 3.32, 95% CI 1.76-6.27).

Shorter breastfeeding duration among CMI and its contributing factors highlighted in this study reflect that living in Ireland does influence Chinese mothers' breastfeeding practices. These findings suggest a need for culturally sensitive and language-specific education and support of breastfeeding among Chinese migrants in Ireland. Cultural beliefs should be aware and acknowledged by health professionals who provide care and support to the Chinese.

PIII-302

Fish Consumption Prior and during Pregnancy of Women in Alberta. Jocelyn E. Graham[1], Laura E. Forbes[1], Casey Berglund[1], Joao Morgadinho[1], Catherine J. Field[1], Rhonda C. Bell[1], The Apron Team[1,2]. *[1]Department of Agricultural, Food and Nutritional Science, University of Alberta, AB, Canada; [2]University of Calgary, AB, Canada.*

Health Canada recommends that pregnant women consume at least 150 grams of cooked fish per week and decrease intake of only specific predatory fish such as tuna, shark and swordfish, which are known to contain high levels of mercury (1). Fish, an important source of omega-3 fatty acids, which may be essential for visual and cognitive development of the fetus. The purpose of this study was to examine women's adherence to guidelines for fish intake during pregnancy and the reasons behind changes in fish consumption as reported by women living in Alberta.

A subgroup of pregnant women (n= 474) from Edmonton and Calgary, Alberta, completed a food frequency questionnaire (FFQ) about dietary habits before pregnancy and a dietary changes questionnaire as part of the Alberta Pregnancy Outcomes for Nutrition (APRON) study. Dietary change in pregnancy was assessed using a questionnaire in which participants recorded foods eliminated, decreased, added or increased since becoming pregnant. Questions regarding fish and seafood intake during pregnancy were analyzed.

Before pregnancy, women consumed a median of 0.61 (Range 0-12) servings of fish per week and 82% did not meet recommendations for fish intake. During pregnancy 85% of women did not meet recommendations with a median intake of 0.51 (Range 0-12) servings of fish per week. Few women (3%) increased or added fish to their diet during pregnancy. Reasons included cravings, health of the baby and for omega-3 or DHA content. Women (36%) reported decreasing or eliminating fish intake due to safety risk, mercury content, nausea and health recommendations. Sushi was the most common seafood item eliminated or decreased by women (n=102).

The majority of pregnant women in Alberta are not meeting the recommendations for fish intake before or during pregnancy. Therefore, few fetuses may be exposed to the beneficial effects of preformed DHA through maternal diet during this essential developmental period. This study highlights the need for better education of safe fish consumption during pregnancy. Further studies will examine the link between maternal omega-3 fatty acid status and cognitive development of offspring.

References

1. Mercury and human health; 2008 [updated November 2008]. Available from: http://www.hc-sc.gc.ca/hl-vs/iyh-vsv/environ/merc-eng.php.

PIII-303

Predictors of Post-Partum Weight Retention and Adiposity at 3 Years in Women in Southern Ontario, Canada. Kim M. Chau[1], Melody Ng[1], Eric Hul[1], Sarah MacDonald[2], Stephanie C. Atkinson[1]. *[1]Pediatrics, McMaster University, ON, Canada; [2]Medicine, McMaster University, ON, Canada.*

We profiled the physical and lifestyle characteristics of pregnant women followed to three years post-partum as part of a birth cohort study to elucidate the key contributors to PPWR.

Demographics, diet by food frequency questionnaire, physical activity by self-report, antidepressant drug use (selective serotonin reuptake inhibitors, SSRI) and anthropometric measurements were assessed during pregnancy and prospectively at three years postpartum in a sample of 233 mothers recruited into the FAMILY birth cohort study. Tricep and subscapular skin fold thickness were measured in mothers in the 3rd trimester, one and three yr postpartum. At three yr, maternal and child adiposity were measured by dual energy x-ray absorptiometry (Hologic Discovery 4500A).

Mothers were 32.8±4.6 yr (mean±SD) at delivery, with 86% of European origin. Smoking occurred in 24% of mothers prior to and 12.6% during pregnancy. The distribution of BMI categories and characteristics of mothers in each category are summarized (mean±SD).

Triceps and subscapular skin fold thickness in mothers rose from pregnancy to 3 yr when the mean weight retention was 2.1 (range - 19.9 to +28.3) kg and whole body % fat was 34.3±7.4%. In a multivariate model for both PPWR at 3 yr and % total body fat, higher GWG and breastfeeding for less than 6 mo were significantly associated with higher PPWR and % body fat. For abdominal % body fat, lowest activity level and having smoked in pregnancy were significant predictors of higher PPWR at 3 yr. The mothers identified as using SSRIs (N=10) compared to an age-matched control group had higher (though not statistically significant) whole body fat (34.5±7.6% vs. 31.0±9.4%) and abdominal fat (30.5±9.1% vs. 25.7±14.5%, NS). As well, SSRI use was associated with greater GWG (14.3±5.9 kg vs. 13.1±4.6 kg control), PPWR (1.28±8.6 kg vs. 0.66±4.6 kg), and % ΔSF thickness from pregnancy to 3 yr post-partum (2.4±32.6% vs. -14.9±21.9%).

In the Hamilton region, > 50% of women enter pregnancy overweight or obese, and also have excessive GWG. The excess GWG and short duration of breast feeding are major determinants of higher PPWR. The use of SSRI anti-depressant drugs may also be a contributing factor. The modifiable risk factors must be considered in planning to reduce GWG in women at risk of excessive weight gains.

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PIII-304

What Do Women Eat during Pregnancy? Vicki L. Clifton[1], Penelope C. McLernon[1], Lisa Wood[2], Vanessa Murphy[2], Michael J. Stark[1], Nicolette A. Hodyl[1]. *[1]Robinson Institue, University of Adelaide, South Australia, Australia; [2]Respiratory Medicine, Hunter Medical Research Institute, New South Wales, Australia.*

Diet and nutrition are essential for a normal healthy pregnancy and evidence suggests that unbalanced consumption of fats, proteins or carbohydrates can impact on fetal growth with long term health implications for the offspring.

Women were prospectively recruited at 12 weeks gestation at the John Hunter Hospital antenatal clinic in Newcastle, Australia. They were Caucasian and from a mixed socioeconomic background. We compared dietary intake of healthy pregnant women (n=47) relative to an asthmatic population (n=84). At 18, 30 and 36 weeks gestation, participants completed a self assessed 24 hour food recall questionnaire. Data was analysed in the Foodworks (Xyris, Brisbane) database, incorporating the AusFoods (Brands), AusNut (All Foods; Food Standards Australia & New Zealand) and RMIT fatty acid databases, from which standard nutrient reference values and fatty acids were extracted. Asthmatic groups were compared based on severity (mild; n=31, moderate/severe; n=53). Data was analysed using SPSS.

There were no significant differences between the groups with respect to maternal age, body mass index (BMI) or total weight gain over pregnancy. All women were overweight based on a BMI >25. There were no significant differences in fetal growth parameters or neonatal outcomes. The mild asthmatic group (n=25) consumed higher quantities of energy, protein, fats, carbohydrates, starch, thiamin, riboflavin, niacin equivalents, magnesium and phosphorous than the moderate/severe asthmatics (n=34) or control population (n=32). When comparing this data to the nutrient reference values for Australia and New Zealand for pregnant women in the third trimester, all women have below average intake of iron, calcium, magnesium, folate, retinol, and dietary fibre. Riboflavin, niacin, vitamin C and A, phosphorous and potassium intake were all above average. Intake of energy and protein were above average in all groups. The total fat intake contributed to 37% of the total energy consumed by the women and 15% of total energy was derived from saturated fats in all groups.

These data indicate that pregnant women consume more nutrients than required for pregnancy and generally consume too much fat in their diet especially saturated fat. They have a significant deficiency in key nutrients derived from fruit and vegetables.

PIII-305

Maternal Asthma Is a Significant Contributor to Neonatal Morbidity. Nicolette A. Hodyl[1], Michael J. Stark[1], Wendy Scheil[2], Vicki L. Clifton[1]. *[1]Robinson Institute, University of Adelaide, South Australia, Australia; [2]Epidemiology Unit, South Australian Department of Health, South Australia, Australia.*

Asthma is the most prevalent chronic condition to affect pregnancies in Australia, currently affecting 12% of pregnant women and expected to rise to 20% in the next five years. We have previously reported an association between maternal asthma and adverse perinatal outcomes, including preterm delivery, still birth and intrauterine growth restriction (IUGR), and other studies suggest an increased risk for congenital malformations. This study aimed to examine the effect of asthma during pregnancy on perinatal birth outcomes and congenital malformation rates in a South Australian cohort.

All singleton birth outcomes in South Australia over ten years (1999-2008; n=178,000) were analysed to assess the effect of asthma on perinatal outcomes. Logistic regression was used to calculate odds ratios and adjust for factors including smoking, maternal age and degree of prematurity.
Asthma was reported in 6.5% of pregnancies, and was associated with a 27% increased risk of preterm delivery (95% CI 1.19-1.36). This effect remained after adjusting for maternal smoking, parity, maternal age and gestational diabetes (OR=1.21, 95%CI 1.13-1.30). Congenital abnormalities were more frequent in pregnancies associated with asthma (3.9% versus 2.4%; p<0.001). An increased requirement for resuscitation (OR=1.15, 95%CI 1.08-1.23) and oxygen therapy >4hours (OR=1.12, 1.03-1.22) was also observed in pregnancies associated with asthma after adjusting for preterm birth, explaining the significant increase in neonatal intensive care admission rates (2.8% versus 2.2%; p<0.001).
An increased risk of preterm delivery and congenital malformations were observed in pregnancies complicated by asthma. Importantly, resuscitation and oxygen therapy were required by neonates of mothers with asthma irrespective of the degree of prematurity. This study has therefore highlighted maternal asthma as a significant contributor to neonatal morbidity.

PIII-306

Maternal Folate and Alcohol Metabolism-Related Gene Polymorphisms and the Risk of Recurrent Pregnancy Loss. Fumihiro Sata[1], Hideto Yamada[2]. *[1]Department of Environmental Health, National Institute of Public Health, Japan; [2]Department of Obstetrics and Gynecology, Kobe University, Japan.*
Epidemiological studies have suggested that the condition might be multifactorial with a possible genetic predisposition and involvement of environmental factors in its pathogenesis. Some environmental factors such as cigarette smoking, alcohol and caffeine consumption have been shown to affect pregnancy outcomes. Among folate and alcohol metabolism-related genes, only associations between genetic polymorphisms in the methylenetetrahydrofolate reductase (*MTHFR*) gene and the risk of recurrent pregnancy loss (RPL) have been often reported, but the results have been inconsistent. The aim of this study is to elucidate the associations between maternal folate and alcohol metabolism-related gene polymorphisms and the risk of RPL.
This case-control study of 87 cases with two or more RPL and fertile 306 controls was performed in the city of Sapporo, Japan. The association between six single nucleotide polymorphisms (SNPs) of folate and alcohol metabolism-related genes, that is, rs1801133 (Ala222Val) and rs1801131 (Glu429Ala) in *MTHFR* gene, rs1805087 (Asp919Gly) in the 5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR*) gene, rs10380 (His595Try) in the 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*) gene, rs1229984 (His48Arg) in the alcohol dehydrogenase 1B (*ADH1B*) gene, rs671 (Glu504Lys) in the aldehyde dehydrogenase 2 (*ALDH2*) gene and RPL was assessed. We performed logistic regression analysis to examine whether there were any associations between each SNP and the risk of RPL.
Without consideration of cigarette smoking or alcohol use, the risk of RPL significantly decreased in the women who had *MTHFR* rs1801133 TT, *MTR* rs1805087 AG or *ALDH2* rs671 AA genotype (p<0.05). With consideration of cigarette smoking and alcohol use, the risk of RPL significantly decreased in women carrying *MTHFR* rs1801133 T allele (odds ratios (OR): 0.51; 95% confidence intervals (CI): 0.27-0.95 [dominant genotype model]). Similarly, the risk of RPL significantly decreased in women carrying the *MTR* rs1805087 G allele (OR: 0.44; 95% CI: 0.23-0.85).
Our findings suggested that the risk of RPL might be decreased due to maternal folate and alcohol metabolism-related gene polymorphisms. Molecular epidemiological studies are further needed to unequivocally elucidate the multifactorial effects of both genetic and environmental factors in the human fecundity.

PIII-307

Plasma Phospholipid Fatty Acid Supply of Expecting Women during Pregnancy and at Delivery: Systematic Review of 102 Articles. Eva Szabo, Tamas Marosvolgyi, Zsofia Steiger, Tamas Decsi. *Department of Paediatrics, University of Pecs, Hungary.*
Long-chain polyunsaturated fatty acids play an important role in the maturation of the developing nervous system. Therefore, our aim was to systematically review available data on fatty acid composition of plasma and erythrocyte membrane lipids in expecting women during pregnancy and at delivery.
Electronic literature search was performed in August 2010, in English (Pubmed, Embase, Cochrane Library, Scopus, ISI Web of Knowledge), German (Springerlink) and Japanese (Journal@rchive) database with the following search expressions: (pregnant OR pregnancy OR gestation OR delivery) AND (arachidonic AND docosahexaenoic) AND human. We analysed data from clinical trials investigating fatty acid composition of plasma or erythrocyte membrane lipids in healthy expecting women, who did not receive fatty acid supplementation during pregnancy.
There were 102 relevant articles publishing fatty acid data of plasma or erythrocyte membrane lipids. Here we focus on plasma phospholipids, which represent an informative marker of the fatty acid status (Fekete K *et al.*, Am J Clin Nutr, 2009). During the first trimester only few studies (n=7) investigated fatty acid status of large (n=3997-7280) populations, while in the three other timepoints there were more studies (n=13-27) investigating somewhat smaller (n=1169-2411) populations. Values of arachidonic acid decreased by the 3rd trimester, while values of docosahexaenoic acid remained quite stable during pregnancy, but decreased by delivery.
1. Our results indicate that on a populational level, docosahexaenoic acid status remains remarkable stable during pregnancy 2. Our present data can serve as reference values for fatty acid supplementation studies in expecting women.

PIII-308

Effect of Uteroplacental Insufficiency on Maternal Later Life Metabolic Disease Risk. Melanie Tran[1], Andrew J. Jefferies[1], Linda A. Gallo[1], Karen M. Moritz[2], Mary E. Wlodek[1]. *[1]Department of Physiology, The University of Melbourne, VIC, Australia; [2]School of Biological Sciences, University of Queensland, QLD, Australia.*
There is a strong inverse relationship between a females own birth weight and her subsequent risk for gestational diabetes. Low birth weight females who develop gestational diabetes during pregnancy also have an increased risk of developing diabetes after their pregnancy in later life. The aim of the study was to determine whether growth restricted females develop metabolic dysfunction after pregnancy in later life.
Uteroplacental insufficiency was induced by bilateral uterine vessel ligation (Restricted) or sham surgery (Control) on day 18 of pregnancy in WKY rats (F0). Control and Restricted F1 female offspring were mated with normal males and allowed to deliver. These females (Ex-Pregnant) were then studied at 13 months and had physiological measures including insulin challenge (1U/kg) to measure whole body insulin sensitivity, intraperitoneal glucose tolerance test (IPGTT, 1g/kg) to determine glucose tolerance and insulin secretion and pancreas collected for determination of β-cell mass, islet number and size. Aged matched Virgin Control and Restricted females were also studied at 13 months with the same physiological measures performed.
Restricted females were born lighter (P<0.05) than Controls and remained lighter throughout the 13 months studied. Absolute, but not relative, pancreatic weight was also reduced at 13 months (P<0.05). Glucose tolerance and first phase insulin secretion was not different between Control and Restricted females in both Virgin and Ex-Pregnant groups. Second phase insulin secretion was reduced in Restricted Virgins (-34%, p<0.05), suggestive of enhanced insulin sensitivity which was confirmed by decreased HOMA-IR (-40%, p<0.05), an index of hepatic insulin resistance. Hepatic insulin resistance increased after pregnancy in both Control and Restricted females (+40%, p<0.05). Pancreatic analysis is ongoing.
Restricted females at 13 months, in both Virgin and Ex-Pregnant groups were more insulin sensitive with no changes in glucose tolerance, suggesting that they are protected from developing metabolic dysfunction. After a pregnancy, Control and Restricted females had increased HOMA, suggestive

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of hepatic insulin resistance that is independent of maternal birth weight. Thus being born small improves insulin action with no effects on metabolic function in later life.

PIII-309

Citrulline Enhances Fetal Growth and Protein Synthesis in a Model of Intra-Uterine Growth Restriction. Aurelie Bourdon, ChristelNowak Nowak, Charlotte Naël, Patricia Parnet, Norbert Winer, Darmaun Dominique. *UMR 1280 Physiology of Nutritional Adaptation, INRA-University of Nantes, Nantes, France.*

Intrauterine growth restriction (IUGR, defined by a birth weight <3rd percentile for gestational age) commonly results from impaired placental blood flow and exposes to increased perinatal mortality and morbidity. Citrulline is a precursor of arginine and NO, which regulates placental blood flow. Moreover, citrulline has been shown to simulate protein synthesis in other models or undernutrition.

To determine whether citrulline supplementation would enhance fetal growth, gestating rats were fed either a control (C; 20% protein) or a low protein (4% protein, LP group) diet. In addition, LP dams were randomized to receive tap water either as such, or supplemented with citrulline (CIT; 2 g/kg/d), arginine (ARG), or an isonitrogenous mix of non essential amino acids (NEAA). On the 20th day of gestation, dams received a 2-hr intravenous infusion of L-[1-13C]valine and L-[1-13C]alanine until fetuses were extracted by C-section. Isotope enrichments were measured in free amino acids and fetal muscle protein by gas chromatography-mass spectrometry.

(means±SD; Student's t-tests): Maternal protein restriction reduced fetal weight (3,81±0,03 and 5,37±0,05g in LP and C, respectively; $p<0.001$). CIT, ARG or NEAA increased fetal weight to 4.12±0.04, 4.00±0.03, and 4,11±0,04g, respectively ($p<0.05$). Plasma fetal/maternal 13C-alanine enrichment ratio, an index of placental alanine transfer, was 0.65±0.43 and 0.36±0.04 in C and LP, respectively (NS). None of the supplements altered this ratio. Fetal muscle protein fractional synthesis rate (FSR) was lower in LP than control fetuses (41±11 vs. 61±13%.d-1, $p<0.001$), and enhanced by CIT (56±4%.d-1), not with ARG or NEAA (45±7 and 50±19%.d-1, NS).

1) citrulline increases fetal growth in a model of IUGR; and 2) the effect may be mediated by enhanced fetal muscle protein synthesis rather than enhanced placental amino acid transfer.

PIII-310

Maternal and Fetal Genotype Is Required to Understand the Full Impact of Genetics on Fetal Growth. Craig E. Pennell[1], Julie A. Marsh[1], Q. Wei Ang[1], H. Rob Taal[2], Lyle J. Palmer[3], Stephen J. Lye[3], Vincent W.V. Jadoe[2], John P. Newnham[1]. *[1]School of Women's and Infants' Health, The University of Western Australia, Perth, Australia; [2]Department of Epidemiology, Erasmus Medical Centre, Rotterdam, Netherlands; [3]Samuel Lunenfeld Research Institute, University of Toronto, Toronto, Canada.*

Genetic variants in the fat mass and obesity-associated (FTO) gene have been shown to be associated with childhood and adult obesity across multiple populations and ethnic groups (N>370,000). Adults homozygous for the minor (A) allele of rs9939609 weigh on average 3-4kg more and have a 1.67-fold increase in the odds of obesity compared with those not inheriting a risk allele. The influence of maternal and fetal FTO polymorphisms on fetal growth is less well understood. *Aim: To investigate the influence of maternal ($_M$) and fetal ($_F$) FTO genotype combinations on fetal growth.*

Data on 1162 singleton births from the Western Australian Pregnancy (Raine) Cohort, with five measures of fetal growth from ultrasound sonography during pregnancy and birth measurements were used for analyses. Femur length, head circumference and abdominal circumference were analysed using linear mixed-effects models, including maternal and fetal genotype and a coded variable for the maternal:fetal genotypic interaction. Birth measurements were analysed using multivariate linear regression.

When maternal or fetal rs9939609 genotypes were considered independently, no associations were detected between maternal or fetal genotype and fetal growth trajectories or birth measurements other than for femur lenth where the $A_{(F)}$ risk-allele was associated with smaller femur growth ($p=9x10^{-4}$). However, when maternal-fetal genotype combinations were considered, the AA_MTA_F and AA_MAA_F were associated with smaller femur length ($p<0.014$), shorter birth length ($p<0.037$) and lower birth weight ($p<0.045$). In addition, the maternal-fetal genotype combinations TA_MTA_F, TA_MAA_F, AA_MTA_F and AA_MAA_F were associated with lower growth trajectories for head circumference ($p<0.0007$) and abdominal circumference ($p<0.004$) with increasing numbers of A alleles progressively increaseing the effect size. In the absence of at least one maternal and one fetal copy of the A risk-allele, no associations with fetal growth trajectories and birth measurements were detected.

These data suggest that both maternal and fetal genotype is required to investigate the genetic influences on fetal growth.

PIII-311

Adverse Health Effects of Prenatal Famine Exposure Are Not Related to Promoter Methylation of Four Candidate Genes. Marjolein V. Veenendaal[1], Paula M. Costello[2], Karen A. Lillycrop[3], Graham C. Burdge[2], Susanne R. de Rooij[1], Rebecca C. Painter[4], Joris A. van der Post[4], Patrick M. Bossuyt[1], Peter D. Gluckman[5], Mark A. Hanson[2], Tessa J. Roseboom[1]. *[1]Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, University of Amsterdam, Netherlands; [2]Academic Unit of Human Development and Health, Faculty of Medicine, University of Southampton, United Kingdom; [3]Faculty of Natural and Environmental Sciences, University of Southampton, United Kingdom; [4]Department of Obstetrics and Gynaecology, Academic Medical Center, University of Amsterdam, Netherlands; [5]Liggins Institute, University of Auckland, New Zealand.*

The Dutch famine birth cohort study has provided the first direct evidence in humans that maternal undernutrition during gestation leads to a 2-fold increase in cardiovascular disease in the offspring. Animal studies have shown that changes in phenotype of the offspring after prenatal undernutrition involve altered epigenetic regulation by DNA methylation. The aim of this study was first to determine whether methylation status of four candidate genes was associated with markers of metabolic and cardiovascular disease. Secondly, we investigated whether methylation status of the proximal promoter regions of four genes differed between individuals exposed to famine at different periods of gestation.

Methylation status of the GR 1-C, PPARγ, LPL and PI3kinase promoters was investigated in DNA isolated from peripheral blood samples of 58 year old subjects born as term singletons in the Wilhelmina Gasthuis in Amsterdam, The Netherlands around the time of the 1944-45 Dutch famine, by methylation sensitive PCR.

Blood pressure, plasma levels of glucose, insulin, triglycerides, LDL, HDL, total cholesterol, diabetes or cardiovascular disease were not associated with methylation status (all $P > 0.05$). Methylation levels did not differ between men and women exposed to famine in either late, mid or early gestation (all $P > 0.05$).

The increased risk of disease after prenatal famine exposure was not associated with differences in average methylation status across the proximal promoter regions of these genes, however, further studies are required to determine whether maternal undernutrition induces specific methylation changes at individual CpGs within the promoter of these genes and whether regions outside the proximal promoter are differently methylated in response to early life environment.

PIII-312

Cord Blood Global DNA Methylation Associated with Childhood Height among Boys. Xiaozhong Wen[1], Ken Kleinman[1], Sheryl L. Rifas-Shiman[1], Andrea Baccarelli[2], Heather H. Burris[3], Augusto A. Litonjua[4], Caroline E. Boeke[5], Matthew W. Gillman[1]. *[1]Dept of Population Medicine, Harvard Med Sch, MA, USA; [2]Ctr of Molecular&Genetic Epi, U of Milan, Milan, Italy; [3]Dept of Neonatology, Beth Israel Deaconess Med Ctr, MA, USA; [4]Dept of Medicine, Brigham&Women's Hosp, MA, USA; [5]Dept of Nutrition, Harvard Sch of Pub Health, MA, USA.*

Few data exist among humans on the roles of DNA methylation in developmental origins of long-term health outcomes. We examined associations of global DNA methylation in cord blood with childhood growth and adiposity.

We analyzed data from 294 boys and 263 girls in Project Viva, a U.S. pre-birth cohort. In venous umbilical cord leukocytes, we used bisulfite-PCR-Pyrosequencing to measure DNA long interspersed nuclear element-1 (LINE-1) methylation (expressed as %5-methylated cytosine). We measured

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children's height/length and weight (6 m, 3 y, and 7y), subscapular (SS) and triceps (TR) skinfold thicknesses (3 y and 7 y), waist circumference (3 y and 7 y), and fat and fat-free mass (7 y) from dual-energy X-ray absorptionmetry (DXA). To account for correlation within individuals, we fit linear mixed models with "LINE-1 × visit" interactions, adjusting for socio-demographics, parental weight and height, gestational weight gain, maternal smoking, gestational age, and birth weight-for-gestational-age z-score.
Boys had higher mean (SD) cord blood LINE-1 methylation than girls (84.8% [0.6] vs 84.4% [0.7]; p-value=<0.0001). At age 7, mean (SD) height z-score in boys was 0.21 (0.99) and in girls was 0.24 (0.93). Associations of LINE-1 with height/length z-score differed by sex. Among boys, the adjusted mean difference in height/length z-score per %increment in LINE-1 was 0.23 (95% CI, 0.07 to 0.40) at 6 m, and 0.24 (0.07 to 0.40) at 3 y and 7 y. Among girls, LINE-1 was not associated with height/length z-score (6 m and 3 y, -0.04 [-0.16 to 0.09]; 7 y, -0.04 [-0.17 to 0.08]). At 7 y, among neither boys nor girls was LINE-1 associated with BMI z-score (0.03 [-0.14 to 0.21] for boys and -0.08 [-0.22 to 0.05] for girls, respectively), DXA fat mass/m2 (0.12 [-0.29 to 0.53] and -0.06 [-0.47 to 0.35]), DXA fat-free mass/m2 (-0.06 [-0.38 to 0.26] and -0.27 [-0.58 to 0.05]), sum of SS + TR, ratio of SS:TR, or waist circumference.
Among boys but not girls, higher LINE-1 (global) DNA methylation in cord blood was associated with greater childhood height up to 7 y. LINE-1 was not associated with adiposity.

PIII-313

The Role of IGF2 and Methylation Regulation in Small for Gestional Age Infants. Shulian Zhang, Chao Chen, Rong Zhang, Wenjing Shi, Yi Dai. *Division of Neonatology, Children's Hospital of Fudan University, Shanghai, China.*
Low birth weight is associated with an increased risk in many diseases in adult life. Insulin-like growth factor II (IGF2) , one paternally expressed gene, plays an important role in fetal and postnatal growth. We investigated the role of IGF2 and the defferentially methylated regions(DMR) status in small for gestional infants.
Plasma IGF2, IGF2R and IGF1 were measured whthin 24 hours after birth in 120 newborn babies, including 30 term appropriate-for-gestational-age(AGA), 30 term SGA, 30 preterm AGA and 30 preterm SGA infants. H19 and IGF2 mRNA level were quantified by fluorescence quantitative PCR. Methylation state of H19 DMR and IGF DMR2 were assessed by using methylation specific PCR (MSP) in these infants.
Plasma IGF2 level after birth is lower in term SGA compared with term AGA(435.11±33.82 vs 620.42±44.79, p=0.002) and preterm SGA infants(435.11±33.82 vs 619.07±44.58, p=0.002). There were significant difference in IGF2 mRNA between preterm SGA vs. preterm AGA(P=0.024) and term SGA vs. preterm SGA(P=0.029). These two H19/IGF2 regions maintain the differential methylation status seen in most other tissues.The level of H19 DMR methylation was higher in term SGA than term AGA infants(P=0.044). There were also significant difference between preterm SGA and preterm AGA(P=0.044). But there were only significant differences in IGF DMR2 between term SGA and preterm SGA(P=0.037).
IGF2 is associated with fetal growth and birth weight. The methylation of H19 and IGF2 may involve in controling IGF2 expression and fetal growth.

PIII-314

Prenatal Protein Restriction Is Associated with Differential DNA Methylation of More Than 200 Promoter Regions in Mice. Mathijs V. Zwier, Agnes Lendvai, Esther M.E. van Straten, Vincent W. Bloks, Torsten Plosch. *Pediatrics, University Medical Center Groningen, Groningen, Netherlands.*
Prenatal nutrition has been epidemiologically identified as a determinant of adult disease. We hypothesized that maternal protein restriction would induce epigenetic adaptations that would interfere with lipid metabolism and hence predispose to atherosclerosis.
C57BL/6 mice were fed a protein restricted diet during pregnancy. CpG island methylation microarrays were performed on fetal liver DNA on day 19.5 of pregnancy. Gene expression was measured by TAQMAN real-time PCR.
204 gene promoter regions were found to be differentially methylated upon protein restriction. *Hyper*methylation and *hypo*methylation were found in comparable numbers. The liver X-receptor (Lxr) alpha promoter was hypermethylated in protein-restricted pups. In parallel, the mRNA level of *Lxra* was reduced by 32% in fetal liver upon maternal protein restriction. Lxr alpha is a nuclear receptor critically involved in control of cholesterol and fatty acid metabolism. In parallel with the reduced expression of Lxra, also the expression of its target genes was significantly reduced in the protein restricted fetuses. *In vitro* methylation of a mouse Lxra-promoter/luciferase expression cassette resulted in a 24-fold transcriptional Lxr repression.
Our study demonstrates that Lxra is a new target of differential DNA methylation and that the Lxr pathway is epigenetically regulated. As Lxr is a key regulator of lipid metabolism, we speculate that these changes in DNA methylation influence plasma lipid levels and thereby contribute to the relationship between early nutrition and adult disease.
Supported by the Netherlands Heart Foundation, grant 2004T048 to T.P.

PIII-315

A Maternal Low Protein Diet Programs Glucose and Fatty Acid Metabolism Differentially in Adult Male and Female Mouse Offspring. Torsten Plosch, Esther M.E. van Straten, Theo H. van Dijk, Vincent W. Bloks, Henkjan J. Verkade, Folkert Kuipers. *Pediatrics, University Medical Center Groningen, Groningen, Netherlands.*
Nutritional conditions during fetal life can influence the risk to develop the metabolic syndrome in adult life ('metabolic programming'). We aimed to establish a mouse model of metabolic programming focusing on the effects of a maternal low protein diet during gestation on glucose and lipid metabolism in the adult offspring.
Pregnant C57BL/6J mice received a control or a low protein diet throughout gestation. Offspring received a low fat diet or a high fat diet from 6-22 weeks of age. Glucose metabolism was studied with a whole-body-glucose test using [6,6-^{2}H]-glucose. Hepatic gene expression was characterized by microarray.
Maternal low-protein diet during gestation led to deteriorated insulin sensitivity upon high-fat feeding in female offspring, as determined by biochemical and microarray analyses. In contrast, female offspring from control diet fed dams was relatively resistant to high-fat diet induced metabolic dysregulation. Maternal low-protein diet did not specifically affect the metabolic parameters addressed in male offspring. In males, the high-fat diet led to insulin insensitivity regardless of the diet of the dam.
Our findings show that fetal malnutrition has limited impact in male mouse offspring, yet it does influence the response to a high-fat diet in females. These findings may have implications for future early diagnostics in the metabolic syndrome and indicates that sex-specific therapies should receive more attention.
Supported by the Netherlands Heart Foundation, grant 2004T048

PIII-316

Lipid Metabolism in Adult Sheep Is Altered by Early Gestation or Post-Weaning Undernutrition. Kirsten R. Poore, Laurence Fulford, Jane K. Cleal, Graham C. Burdge, Mark A. Hanson, Lucy R. Green. *Institute of Developmental Sciences, University of Southampton, United Kingdom.*
In adult sheep, post-weaning undernutrition (UN) improved glucose tolerance in females via up-regulation of insulin signalling in skeletal muscle, but not in adipose tissue, whereas in males early gestation UN increased adipose tissue lipoprotein lipase mRNA expression [1,2]. Both effects may predispose to later obesity. We further investigated the regulation of lipid metabolism by insulin in these animals by measuring plasma triacylglycerol (TAG) and non-esterified fatty acid (NEFA) concentrations during a glucose tolerance test (GTT).
Ewes received either 100% (C, *n*=27) or 50% nutritional requirements (U, *n*=29) from 1-31 days gestation and 100% thereafter. Male and female offspring were then fed either *ad lib* (CC, *n*=13; UC, *n*=15) or exposed to dietary restriction to reduce body weight to 85% of target from 12–25 wk age (CU, *n*=14; UU, *n*=14) and *ad lib* thereafter. Plasma NEFA and TAG concentrations were measured by an automated colorimetric assay and gas chromatography, respectively, during GTT at 1.5 and 2.5 yr. Summary measures (baseline, slope, maximum fall from baseline and area under curve relative to baseline (AUC)) were analysed by ANOVA.

The fall in plasma TAG during GTT at 2.5 yr was greater in postnatally undernourished compared to control sheep (10.8±1.8 *vs.* 7.6±1.0 μg/ml; $P<0.05$), regardless of prenatal nutrition. The fall in plasma NEFA during GTT at 2.5 yr was less in prenatally undernourished compared to control sheep (AUC: 28613±1896 *vs.* 36808±3152 μmol.min/l, $P<0.05$; slope 10-30 min: -9.4±0.9 *vs.* -7.2±1.4 μmol/l/min, $P<0.1$), regardless of postnatal nutrition. These effects were not observed at 1.5 yr, nor affected by sex.
The effects on plasma TAG and NEFA profiles during GTT suggest enhanced insulin sensitivity in adipose tissue and/or liver following postnatal UN, but reduced effectiveness of insulin to inhibit adipose tissue lipolysis following early gestation UN. These effects only became apparent in older sheep and may constitute two developmental pathways to increased fat deposition. Together with sex-specific dysregulation of associated mechanisms in these animals [1,2], their risk of obesity and dyslipidaemia may be increased, particularly in the face of an over-abundant diet.
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[1] Poore *et al.*. 2007 *Am J Phys* 292: E32; [2] Poore *et al.*. 2009 *J DOHaD* 1 S1 212.

PIII-317

The Intersection of Fatty Acid Oxidation, Diet-Induced Insulin Resistance and Epigenetics. Jennifer K. Raymond[1], Melanie B. Gillingham[2]. *[1]Department of Pediatrics, Oregon Health and Science University, Portland, OR, USA; [2]Department of Molecular and Medical Genetics, Oregon Health and Science University, Portland, OR, USA.*
The role of fatty acid oxidation (FAO) in the development of insulin resistance is debated. Two opposing theories are the lipotoxicity hypothesis and the mitochondrial dysfunction hypothesis. The goal of this study was to further examine the role of decreased FAO in the development of diet-induced insulin resistance in mice with a homozygous deletion of very long-chain acyl-CoA dehydrogenase (VLCAD) compared to wild type mice after 12 weeks of high-fat diet.
Experiment 1: VLCAD knock out (-/-) and VLCAD wild type (+/+) mice from homozygous breeding pairs were placed on high-fat mouse chow. After 12 weeks, body composition by NMR, energy expenditure by indirect calorimetry and glucose clearance by glucose tolerance test (GTT) were measured. Experiment 2: The same studies were repeated in VLCAD -/- and VLCAD +/+ littermates from heterozygous breeding pairs.
VLCAD +/+ male offspring with VLCAD +/+ homozygous parents (n=4) had the greatest weight gain from baseline compared to VLCAD -/- males with VLCAD -/- homozygous parents (n=6) and VLCAD -/- and VLCAD +/+ males with VLCAD +/- heterozygous parents (n=9 and n=6, respectively).
VLCAD -/- males with VLCAD -/- homozygous parents had significant improved glucose clearance following GTT when compared to VLCAD +/+ males with VLCAD +/+ homozygous parents (p-value = 0.027). VLCAD -/- males with VLCAD +/- heterozygous parents had similar glucose clearance following dextrose bolus compared to VLCAD +/+ male littermates (p-value = 0.534).
VLCAD +/+ mice from homozygous parents were more susceptible to increased weight gain and decreased glucose clearance following high-fat diet when compared to VLCAD -/- mice from homozygous parents and VLCAD +/+ and VLCAD -/- from heterozygous parents. Intra-uterine exposure to increased free fatty acids, changes in glucose and differences in maternal stress may be impacting adult offspring. Differences in offspring of homozygous VLCAD+/+, homozygous VLCAD-/- and heterozygous VLCAD +/- matings suggest an epigenetic effect rather than the single gene deletion on the adult offspring's susceptibility to diet-induced glucose intolerance.

PIII-318

The Relationship between C-Reactive Protein and Birth Weight, the Nutritional Status, Lipid Profile and Insulin Sensitivity of Brazilian Children. Patricia H. Rondó[1], Jesuana O. Lemos[1], Joilane A. Pereira[2], Rosimeire Ferreira[1]. *[1]Nutrition, University of Sao Paulo, Sao Paulo, Brazil; [2]Nutrition, Federal University of Piaui, Rua Cicero Eduardo s/n, Teresina, Piaui, Brazil.*
To assess the relationship between high-sensitivity CRP (hs-CRP) levels in children and markers of their nutritional status at birth and in childhood, dyslipidemia and insulin sensitivity.
Prospective cohort study involving 495 children from five to eight years of age. Birth weight was informed by the mothers, body mass index and waist circumference were determined. hs-CRP was measured in plasma by latex-enhanced nephelometry. Total cholesterol and HDL-c were determined by a colorimetric enzymatic method. Triglycerides were asssessed photometrically and insulin was assayed in serum by chemiluminescence. Insulin resistance was determined by the homeostasis model assessment – HOMA method.
The impact of the nutritional status, dyslipidemia, insulin sensitivity and other independent variables on hs-CRP was determined by linear regression using the backward stepwise selection method. There were negative statistically significant associations between hs-CRP and age ($p<0.001$) and HDL-c ($p=0.006$), and positive associations between hs-CRP and waist circumference ($p<0.001$) and gender ($p<0.001$).
It is difficult to interpret the association between hs-CRP and age, considering the close age range of the children included in the study. Confirming the results of other studies our results showed associations between hs-CRP and the lipid profile and female gender. The association between hs-CRP and waist circumference imply that abdominal adiposity, even in very young ages is a matter of concern. A limitation of our study is the use of a single hs-CRP measurement, which may not accurately reflect long-term inflammation status. Therefore, the authors advise future studies to confirm their results.

PIII-319

Protein Malnutrition during Adolescence Causes Irreversible Changes on Glucose Homeostasis in Adult Rats. Pamelli M.S. Silva[1], Dionízia X. Scomparini[1], Ana C.M. Lazzari[1], Aryane R. Agostinho[1], Rodrigo M. Gomes[1], Sabrina Grassiolli[2], Clarice Gravena[1], Paulo C.F. Mathias[1], Fernanda N.S. Almeida[1]. *[1]Department of Cell Biology and Genetics, State University of Maringá, Paraná, Brazil; [2]Department of Biology, State University of Ponta Grossa, Paraná, Brazil.*
This study investigates whether a protein restriction during adolescence is capable of programmed metabolically adult animals.
Rats were treated with a diet low in protein (4%) during adolescence (LP-group). Control rats (NP-group) fed diets with normal protein (23%). At 120-day-old the animals were subjected to glucose tolerance test (ivGTT) and insulin tolerance test (ITT), after those biometric parameters was also evaluated.
The administration of low-protein diet during adolescence 30 days attenuated the accumulation of adipose tissue. There was a slight increase in glucose levels at 5min in LP animals compared with NP ones. When we calculated the area under the glycemic curve during the ivGTT observed that blood glucose levels increased 25% in LP than in NP animals. Insulin sensitivity was indirectly assessed by calculating the HOMA index and K_{itt}. Both index were able to demonstrate a increasing by 40% in insulin sensitivity in LP rats compared to NP ones.
Our study showed that malnutrition during adolescence led to a reduction in fat stores, hypoinsulinemia, mild glucose intolerance offset by a high peripheral insulin sensitivity and fasting normoglycemia.
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PIII-320

Lower Resting Energy Expenditure at Adult Age in Higher Birth Weight Babies. Tammy Lin Lin Song[1], Kavita Venkataraman[2], Yap Seng Chong[2], Peter Gluckman[3], Eric Yin Hao Khoo[1], Chin Meng Khoo[1], Melvin Leow[3], Yung Seng Lee[4], Paul Deurenberg[2], E. Shyong Tai[1]. *[1]Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [2]Obstetrics & Gynaecology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [3]Singapore Institute of Clinical Sciences, Singapore; [4]Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.*

Previous studies have suggested that high birth weight was associated with changes in body composition and energy metabolism that may predispose an adult individual to obesity. The objective of this study was to examine the relationship between reported birth weight and body composition and resting energy expenditure (REE) in adult life.

One hundred and twenty overweight and obese but otherwise healthy men of different ethnic groups (44 Chinese, 44 Malay and 32 Indian), aged 21 to 40 years, body mass index (BMI) of 23.0 to 30.0 kg/m^2 with no recent change in weight and normal thyroid function tests were recruited in this cross sectional study. Self reported birth weight was categorized based on quartiles as lower birth weight (LBW, $\leq 25^{th}$ percentile, 2.8 ± 0.1 kg, n = 31), normal birth weight (NBW, 3.2 ± 0.1 kg, n = 60) and higher birth weight (HBW, $\geq 75^{th}$ percentile, 3.6 ± 0.2 kg, n = 29). REE was measured with an indirect calorimetric ventilated hood system (Quark CPET, COSMED) and body composition was measured by dual energy X-ray absorptiometry (Hologic Discovery Wi).

There was no significant relationship between birth weight and adult weight, height, BMI, total fat free mass (FFM) and total fat mass adjusted for ethnicity. However, birth weight was negatively correlated with REE adjusted for total FFM, total fat mass and ethnicity ($r = -0.18$, $p < 0.05$). After adjustment for total FFM, total fat mass and ethnicity (ANCOVA), the HBW group had a 99 ± 45 kcal/day lower REE ($p<0.05$) compared with the LBW group whereas no significant difference was observed between the NBW and HBW groups.

Birth weight is a significant predictor of REE, independent of ethnicity, total FFM and total fat mass. This suggests that foetal programming affects REE in adulthood by mechanisms independent of body composition.

PIII-321

Identification of New Targets for Insulin Resistance and Increased Glucose Production in the Growth Restricted Fetal Sheep Liver. Stephanie R. Thorn, Laura D. Brown, Paul J. Rozance, William W. Hay, Jacob E. Friedman. *Pediatrics, University of Colorado, CO, USA.*

Recent data indicate that fetal sheep with intrauterine growth restriction (IUGR) have increased glucose production and gluconeogenic gene expression in late gestation that are not suppressed by acute hyperinsulinemia. Development of hepatic insulin resistance and glucose production during fetal life could predispose IUGR offspring to unregulated glucose production and diabetes in later life. Our aim is to identify targets and pathways involved in the regulation of insulin sensitivity and induction of gluconeogenesis in the IUGR fetal liver.

We performed transcript profiling (Affymetrix arrays, bovine) using RNA from late gestation (135 days) livers from control (CON) and IUGR fetuses receiving saline and CON and IUGR fetuses during hyperinsulinemic-euglycemic clamp (INSULIN, 3h). Data were analyzed by ANOVA with effects of IUGR (CON, IUGR), INSULIN (saline, insulin), and interaction (IUGR x INSULIN) using GeneSpring GX11 (Agilent). Gene ontology (GO) analysis was performed to identify gene function.

IUGR had a significant effect on the hepatic transcriptome. GO analysis identified significant gene groups relating to oxidative metabolism, lipid biosynthesis, glucose metabolism, cofactor binding, and chemokine activity. These expression patterns support coordinated changes in IUGR hepatic metabolism including deceased lipid synthesis, increased urea cycle activity, decreased acetyl CoA and TCA cycle activity, and increased lactate utilization and gluconeogenesis. We also identified increased expression of transcriptional (PGC1α, ATF3, CREM) and signaling (PTPRD, TRB3) regulators that may be involved in the early activation of gluconeogenesis in IUGR. As expected, INSULIN significantly and similarly regulated many genes in both CON and IUGR livers. Importantly, we identified a group of genes with a significant IUGR x INSULIN interaction. These genes are candidates for the development of hepatic insulin resistance in IUGR and include transcription factors and regulators of cell signaling (SGK1, JUN, ERBB3).

IUGR results in major effects on hepatic metabolism, including increased gluconeogenesis. We have identified new targets that may be responsible for the induction of this pathway in utero. These targets provide insight into the mechanisms for fetal programming of diabetes and for developing strategies to improve health outcomes in IUGR offspring later in life.

PIII-322

Maternal High-fat Diet before and during Pregnancy Leads to Hypertension and Impaired Glucose and Lipid Metabolism in the Adult Mouse Offspring. Takashi Umekawa, Takashi Sugiyama, Du Qinwen, Norimasa Sagawa. *Obstetrics and Gynecology, Mie University Graduate School of Medicine, Japan.*

In order to clarify the role of maternal excess fat intake in the fetal programming, we have investigated the effects of a maternal high-fat diet before and during pregnancy on blood pressure and glucose and lipid metabolism in offspring.

C57BL/6 female mice were fed either a control (C) diet (10% fat) or a high-fat (HF) diet (45% fat) for six weeks before mating and during pregnancy. Pups were culled or moved to other dams fed the C diet to exclude the influence of maternal diet during lactation. Male offspring were weaned on to the C diet at three weeks of age and blood pressure and glucose and lipid metabolism were assessed until 28 weeks of age.

The daily calorie intake from fat in the HF diet mice was 4-5 times as high as that in the C diet mice before and during pregnancy. Although the cumulative food intake of the HF diet offspring after weaning was lower than that of the C diet offspring (2262.6 ± 31.1 vs. 2134.6 ± 20.6 kcal, $p<0.05$), there were no significant differences in body weight between the two groups during the experimental period. Their blood pressure, glucose tolerance test and insulin tolerance test (ITT) showed no significant differences between both groups at 10-12 weeks of age. In contrast, the HF diet offspring had notably higher serum free fatty acid (FFA) and triglyceride (TG) levels after 24 hours of refeeding following a 24-hour fast at 21 weeks of age (FFA: 0.62 ± 0.05 vs. 0.38 ± 0.03 mEq/l, $p<0.05$. TG: 159.0 ± 13.9 vs. 83.0 ± 13.5 mg/dl, $p<0.05$). Moreover, the HF diet offspring showed significantly higher blood pressure than the C diet offspring at 25 weeks of age (114.7 ± 1.3 vs. 99.3 ± 2.9 mmHg, $p<0.05$) and exhibited impaired glucose tolerance at 26 weeks of age. There was no significant difference found, however, in ITT between both groups at 28 weeks of age.

These findings indicate that a maternal high-fat diet before and during pregnancy predisposes their offspring to hypertension and to the disturbance of glucose and lipid metabolism in adult life, even without the presence of obesity.

PIII-323

Neonatal Overexposure to Natural Glucocorticoids Programs Insulin Sensitivity in Yearling Ponies. O. A. Valenzuela, J. K. Jellyman, N. B. Holdstock, V. L. Allen, A. J. Forhead, A. L. Fowden. *Deparment of Physiology, Development and Neuroscience, University of Cambridge, Cambridgeshire, United Kingdom.*

Prenatal overexposure to synthetic glucocorticoids (GCs) is known to programme adult metabolic phenotype. Much less is known about the programming effects of natural GCs, particularly in the neonatal period. This study examined the effects of neonatal cortisol overexposure on insulin sensitivity (IS) of juvenile ponies.

After normal term delivery, pony foals were treated intramuscularly with saline (S, n=8, four of each sex) or ACTH twice daily (n=9, four colts & five fillies, 0.125mg Depot Synacthen) for five days to raise plasma cortisol to values seen in ill neonates. Jugular blood samples were taken daily during treatment. At 15±1 and 30±1 months, catheters were inserted into the aorta and vena cava under general anaesthesia. After 14 days recovery and fasting overnight, IS was measured by hyperinsulinaemic-euglycaemic clamp. Insulin was given continuously (5mU/min/kg) for 2-5h before glucose levels were clamped at pre-infusion values by infusing 40% glucose. Blood samples were taken at 10min intervals for 40min before insulin infusion and again at clamped steady state. The glucose infusion rate during the clamp

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corrected for body weight was used as the measure of IS. All procedures were licensed by the UK Home Office. Statistical significance was assessed by t-test or two-way ANOVA (treatment and sex).

Plasma cortisol levels were eight-fold higher in ACTH than S treated foals during treatment. Basal blood glucose concentrations were similar in the two treatment groups as 1 (S, 4.32±0.24mmol/l, n=8; ACTH, 4.71±0.23mmol/l, n-=5) and two year olds (S, 4.27±0.21mmol/l, n=8; ACTH, 4.85±0.30mmol/l, n=7). In yearlings, IS was greater in ACTH (23.3±2.3µmol/min/kg, n=8) than S treated animals (17.1±2.0µmol/min/kg, n=5, p<0.02). Yearling fillies were also more insulin sensitive than colts (Fillies, 26.2±2.1µmol/min/kg, n=6; Colts, 16.4±1.1µmol/min/kg, n =7), irrespective of treatment (P<0.002). Neither treatment nor sex affected IS in the two year olds (P>0.05). However, IS at this age was lower than yearling values in ACTH (14.7±1.9µmol/min/kg, n=7, p<0.01) but not S treated animals (17.5±1.8µmol/min/kg, n=8, p>0.05).

ACTH-stimulated cortisol overexposure of newborn foals increased their IS as yearlings but not as two year olds. Natural GCs within the physiological range can, therefore, programme metabolic phenotype neonatally.

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PIII-324

Is the Fetal Origins Hypothesis of Diabetes Supported by Animal Research? A Systematic Review and Meta-Analysis of the Evidence.

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The fetal programming hypothesis states that fetal undernutrition during pregnancy results in permanent changes in the offspring's metabolism. A large number of animal studies have evaluated the effect of fetal undernutrition on later susceptibility to type 2 diabetes with varying results.

The aim of this study is to systematically review the existing animal literature examining effects of prenatal undernutrition on glucose and insulin metabolism.

An electronic search was performed in Medline and Embase to identify all articles that reported studies investigating the effect of fetal undernutrition on plasma insulin, plasma glucose and beta cell mass in animal models. Summary estimates of the effect of undernutrition on mean glucose concentration, insulin level, and beta cell mass were obtained through meta-analysis.

The search resulted in 1827 articles, of which 117 were potentially eligible, based on title and abstract, and 49 met the selection criteria and were included in the review. Prenatal protein restriction increased plasma glucose concentrations (0.42 mmol/l (95% CI 0.07 to 0.77)). Both general undernutrition and protein restriction reduced plasma insulin concentrations (general undernutrition: -0.03 nmol/l (95%CI -0.04 to -0.01), protein restricted: -0.04 nmol/l (95%CI -0.08 to 0.00)) and beta cell mass (general undernutrition: -1.24 mg (95% CI -1.88 to -0.60), protein restriction: -0.99 mg (95% CI -1.67 to -0.31)). In all cases, heterogeneity was significant.

The available evidence from experiments in different species shows that prenatal undernutrition – both general or protein restriction – results in increased glucose and reduced insulin concentrations as well as beta cell mass in later life.

PIII-325

Programmed Increase in Adipose Tissue Fatty Acid De Novo Synthesis and Desaturation.

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Small-for-gestational age (SGA) at birth is a risk factor for adult obesity. Maternal food-restriction during rat pregnancy results in SGA pups who demonstrate catch-up growth and increased insulin sensitivity (lower glucose and insulin levels) after normal nursing, but become insulin resistant, obese adults. Abnormal lipogenesis is implicated in the mechanisms of obesity development. Acetyl-CoA carboxylase 1 (ACC1) catalyzes the rate-limiting step in fatty acid de novo synthesis, and stearoyl-CoA desaturase enzyme 1 (SCD1) converts saturated to monounsaturated fatty acids for storage in triglycerides. Carbohydrate-response element binding protein (ChREBP) is activated by carbohydrate intake and is known in liver to promote expression of ACC1 and SCD1. Its role in programmed adipose tissue lipogenesis is unknown. The aim of the study is to determine adipose tissue de novo synthesis, desaturase activity, and ChREBP expression in young SGA offspring.

Control dams received ad libitum food from day 10 to 21 of gestation, and study dams were 50% food-restricted to produce SGA pups. Control dams nursed all pups and male offspring were studied at age 3 weeks. Dams received 6% deuterium as a stable isotope tracer in drinking water. Fatty acid de novo synthesis rates and the desaturation index were determined by gas chromatography/mass spectrometry in subcutaneous adipose tissue. Protein expression of ACC1 and ChREBP were determined by Western blotting. mRNA expression of SCD1 was determined by real-time PCR.

SGA and Control offspring weighed similarly. SGA offspring exhibited increased palmitate de novo synthesis (43.5%±1.1 versus 40.0%±1.4, p<0.05) and increased desaturation index (3.51±0.001 versus 3.23±0.005, p<0.05) compared to Controls. SGA demonstrated strong trends toward increased ACC1 protein expression (1.4 fold) and SCD1 mRNA expression (1.65 fold). ChREBP protein expression was significantly increased in SGA (1.4-fold, p<0.05).

Increased de novo synthesis and desaturation are present in SGA adipose tissue at three weeks of age. These changes are accompanied by increased expression of ChREBP. The increased expression of ChREBP is present despite lower glucose levels at this age. These findings suggest a role for ChREBP upregulation in programmed abnormal lipogenesis.

PIII-326

Are There Changes in DNA Methylation in Response to Chronic Consumption and Withdrawal of Folic Acid in a Population-Based Trial of Reproductive Age Women?

Krista S. Crider[1], Robert J. Berry[1], Christopher J. Bean[1], Thomas P. Yang[2], Jason O. Brant[2], Sonja A. Rasmussen[1], Ling Hao[3], Zhu Li[3], David R. Maneval[2], Eoin P. Quinlivan[2], Lynn B. Bailey[2]. *[1]NCBDDD, CDC, USA; [2]University of Florida, FL, USA; [3]Peking University, China.*

We evaluated the effect of folic acid (FA) supplementation and withdrawal on DNA methylation as part of a population-based trial.

Northern Chinese women (n=96) of childbearing age were enrolled in a six month supplementation trial of different FA doses: 100, 400, 4,000 µg/d and 4,000µg/wk followed by a three month withdrawal. Blood samples were screened for changes in DNA methylation level and patterns at five time points: 0, 1, 3 and 6 months supplementation and after a three month withdrawal. We utilized liquid chromatography tandem mass spectrometry to determine the ratio of methyl cytosine to cytosine to determine global methylation levels as well as bead array technology to examine 1505 loci in 802 genes.

No changes from baseline were found in global methylation level even after six months of 4,000 µg/d FA. There was no association of methylenetetrahydrofolate reductase (*MTHFR)* genotype with global methylation level. Mean baseline site specific methylation patterns were highly correlated among the three *MTHFR* genotypes ($r^2 > 0.99$). At baseline > 99.4% of these loci had little variation in mean betas (ratio of methylated/unmethylated), with between -5 and +5% difference between the three *MTHFR* genotypes. After supplementation, the variation among

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individuals in locus-specific DNA methylation levels increased compared to baseline. CpG sites that showed significant mean change ($p<0.01$) from baseline to any time point were more likely to be part of non-CpG islands vs. CpG islands and from loci on the X vs. autosomes. There were more loci with significant changes ($p<0.01$ and change in % methylation of >+/-10) from baseline at any point during supplementation among those with either the variant TT (6.6%) or heterozygous CT (4.5%) genotype compared with the CC (2.5%) genotype. After withdrawal of FA there were limited numbers of loci with significant differences from baseline (CC 0.5%, CT 0.9% and TT 0.7%).

There were no dramatic and consistent long term change in DNA methylation in response to FA supplementation and withdrawal in either global DNA methylation level or at specific genetic loci, however; specific CpG sites may be more susceptible to increased variability in methylation in response to FA exposure.

PIII-327

Consequences of Folate Depletion during Development for DNA Methylation and Gene Expression in the Fetal Mouse. Jill A. McKay[1], Dianne Ford[1], Caroline L. Relton[1], Chris T. Evelo[2], Michiel Adriaens[2], John C. Mathers[1]. *[1]Human Nutrition Research Centre, Newcastle University, Tyne and Wear, United Kingdom; [2]BiGCaT, Maastricht University, Netherlands.*

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. Epigenetic markings, including DNA methylation patterns, are one potential mechanism mediating these effects. Since folate is a methyl donor, altered folate supply may influence methyl group availability for DNA methylation. Indeed, we observed previously that genomic DNA methylation was lower in adult offspring born to folate depleted dams ($p=0.010$, McKay *et al.* 2011). Furthermore, maternal folate depletion altered methylation in a gene specific manner in the fetal gut (McKay *et al.*, submitted). In this study we investigated the influence of maternal folate depletion on genome wide DNA methylation, and on gene expression, in the male fetal liver.

Pairs of female C57BL/J6 mice were assigned randomly to a folate-adequate (2 mg folic acid/kg; Control) or folate-deplete (0.4 mg folic acid/kg; Test) diet four 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. DNA and RNA were extracted simultaneously from fetal livers.

To identify genes regulated by maternal folate depletion, hepatic RNA from male fetuses was hybridized to NuGO Affymetrix mouse whole genome expression arrays. To identify regions of the genome that were differentially methylated between Test and Control groups, methylated DNA was immunoprecipitated, then amplified by PCR, before hybridization to Roche NimbleGen Methylation 385K arrays.

In male fetal livers, 679 genes were differentially expressed (321 up-regulated, 358 down-regulated; fold change of +/-1.2 and $p<0.05$) in response to maternal folate supply. Preliminary analysis showed that in both Test and Control groups, 1343 gene promoters were highly methylated in the fetal liver. A further 599 promoters were highly methylated in response to low folate only, whereas 338 promoters were highly methylated in the control group only. Twenty six genetic loci were both differentially expressed and differentially methylated between Test and Control groups.

We are exploring the influence of epigenetic regulation of the altered gene expression observed.

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PIII-328

Folate Depletion during Development Causes Gene Expression Changes in Fetal and Adult Mouse Liver. Jill A. McKay[1], Michiel Adriaens[2], Long Xie[1], Chris T. Evelo[2], Caroline L. Relton[1], Dianne Ford[1], John C. Mathers[1]. *[1]Human Nutrition Research Centre, Newcastle University, Tyne and Wear, United Kingdom; [2]BiGCaT, Maastricht University, Netherlands.*

Nutritional insults *in utero* can result in altered programming of offspring, leading to increased adulthood disease risk. Epigenetic markings, including DNA methylation, are one potential mechanism mediating these effects via consequent gene expression changes. Folate depletion may influence DNA methylation, and therefore gene expression, through its effects on the supply of the methyl donor S-adenosylmethionine. Indeed, we observed lower genomic DNA methylation in adult offspring born to folate depleted dams ($p=0.010$, McKay *et al.* 2011) and altered gene specific methylation in the fetal gut in response to folate depletion (McKay *et al.*, submitted). In this study we examined the influence of maternal folate depletion on gene expression in the male fetal and adult liver to investigate the functional effects of reduced folate intake during pregnancy and the sustainability of these effects in later life.

To obtain fetal liver samples, female C57BL/J6 mice were assigned randomly to a folate-adequate (2 mg folic acid/kg; Control) or folate-deplete (0.4 mg folic acid/kg; Test) diet four weeks prior to timed mating. Dams remained on allocated diets until day 17.5 gestation, when dams were killed and fetal livers removed. To obtain adult liver samples, female C57BL/J6 mice were assigned randomly Control or Test diets four weeks prior to mating and remained on allocated diets during pregnancy and lactation. Offspring were fed normal folate diets post-weaning. Mice were killed at 6.5 months and livers removed. Hepatic RNA from male fetal and adult mice (n = 12 for each group) was hybridized to Affymetrix mouse whole genome arrays.

Gene expression in the liver during fetal development appeared to be more tightly controlled than in the adult. Maternal folate depletion resulted in 679 differentially expressed genes (321 up-regulated, 358 down-regulated) in fetal liver compared with 3351 differentially expressed genes (2088 up-regulated, 1279 down-regulated) (fold change of +/-1.2 and $p<0.05$) in adult liver. Of these, 101 genes were expressed differentially in both fetal and adult livers and 60 genes demonstrated the same directional change.

This observation may indicate sustained gene expression changes caused by folate depletion during development. Further analysis of data generated from these analyses will be presented.

PIII-329

Taurine (Tau), a Micronutrient Required for Pancreatic β-Cell Development, Is Reduced in Fetal (F) but Not Maternal (M) Blood in Baboon Pregnancies in Both Moderate Global Maternal Nutrient Restriction (MNR) and Nutrient Excess (MNE) – A Commonality of Programming of Offspring (OFF) Predisposition to Diabetes? Peter W. Nathanielsz[1], Thomas J. McDonald[1], Guoyao Wu[2], Mark J. Nijland[1]. *[1]Center for Pregnancy and Newborn Research, Dept OB/GYN, The University of Texas Health Science Center San Antonio, TX, USA; [2]Dept. Animal Science, Texas A&M University, TX, USA.*

OFF of rats fed both low protein diets (LPD) and high energy MNE diets in pregnancy and lactation exhibit a predisposition to diabetes in adult life (ref). MNR in baboon pregnancy decreases F pancreatic islet size and number and insulin content (Reproductive Sciences 15 122A: 2008) and results in an early prediabetic state in OFF at 3.5 years of age (unpublished). In both MNR and MNE there is decreased placental amino acid transport. Decreased Tau availability may be a factor in common, since Tau in drinking water of rats reverses OFF metabolic outcomes resulting from LPD (Diabetologia 2008 51:836; J. Physiol. 2007 5;823). We hypothesized that reduced fetal Tau availability is common to both MNR and MNE, and may constitute a common mechanism of impaired pancreatic development in primates.

All females were lean and normal weight for age at the start. CTR (n = 22) baboons ate normal chow (CTR - 12% energy from fat with 0.29% glucose and 0.32% fructose; n=22) throughout. MNR (n = 6) ate 70% CTR from 0.16 to 0.9 G. MNE were fed a diet with 45% energy from fat with 4.62% glucose and 5.64% fructose plus free access to fructose beverage for at least nine months prior to pregnancy (n=5), MNE mothers had increased M triglycerides and body fat. Fetuses were removed by CSection at 0.9 G under general anesthesia and M and F blood obtained for measurement of plasma Tau by HPLC. Data mean ± SEM; Student's t-test; $p < 0.05$.

Maternal Tau was unchanged in the three groups (151 ± 1.3 μM (CTR), 122 ± 8.3 μM (MNR) 120 ± 16.7 μM (MNE) while fetal Tau was decreased in both MNR and MNE compared with controls $p < 0.05$; 206 ± 12.1 μM (CTR) 140 ± 17.5 μM (MNR); 125 ± 4.2 μM (MNE).

Fetal Tau was similarly reduced in both MNR and MNE at 0.9 G indicating a potential common mechanism involved in impaired pancreatic development that leads to a predisposition to diabetes. In both the setting of MNR and MNE. Since fetal to maternal Tau ratio was reduced, we propose that part of the mechanism is that placental Tau transport is decreased in both MNR and MNE conditions.

PIII-330

Fetal Vitamin B12 Is Similarly Decreased in Baboon Pregnancy in Both Maternal Nutrient Restriction (MNR) and Maternal Obesity Combined with Maternal Nutrient Excess (MO/MNE). However Changes in Placental B12 Transcobalamin Receptor (TCN2R) Immunoreactivity (IR) Differ in These Two Situations. Peter W. Nathanielsz, Jaehyek Choi, Mark J. Nijland, Cun Li. *Center for Pregnancy and Newborn Research, Dept OB/GYN, The University of Texas Health Science Center San Antonio, San Antonio, USA.*

We have previously reported that fetal B12 is decreased when pregnant baboons eat either a restricted diet (70% controls) or an MNE diet producing MO levels in the presence of MNR (Mitsuya K *et al.* Reprod. Sci. 17(3 Suppl): 107A. Ab.151). We hypothesize that this dysfunction of the one carbon cycle (1-CC) represents a commonality resulting in the epigenetic changes underlying developmental programming in these two different nutritional challenges. At 0.9 gestation we determined placental transcobalamin receptor (TCN2R) immunoreactivity (IR) in placentas of pregnant baboons that had been on the control or MNE diet before and during pregnancy and compared the findings with our previous observations in MNR.

Healthy female baboons of similar body weight ate normal chow (CTR - 12% energy from fat; 0.29% glucose,0.32% fructose; n=15) or MNE - 45% energy from fat; 4.62% glucose and 5.64% fructose plus free access to fructose sodas for at least nine months prior to pregnancy) that increased maternal body fat and triglycerides (n=5). Fetuses were removed by CSection at 0.9 G under general anesthesia and placental TCN2R protein IR was measured (R&D Systems, antibody AF1557). Density and fraction stained were quantified with Image J program (NIH). Statistical analysis. Significance was set to $p<0.05$ (unpaired t-test).

Placental IR TCN2R was unchanged in MO/MNE: 92.79 $\pm$ 9.06 vs79.86 $\pm$ 5.01 (CTR) for density and fraction stained 31.65$\pm$ 2.96 (MNE) and 27.16$\pm$ 1.56(CTR).

The mechanisms producing the very similar falls in fetal B12 in MNR and MNE differ. While placenta IR TCN2R is unchanged in MNE it is reduced in MNR. NCRR P51 13986. R24RR21367.HD 21350,

PIII-331

A Study of Vitamin (OH) D3 Status in Patients with Diabetes Mellitus Type 2. Hetal Parekh, Anuradha S. Shekar. *Food Science and Nutrition, Dr. BMN College of Home Science, Maharashtra, India.*

AIMS AND OBJECTIVES:

• To study the prevalence of Vitamin D deficiency in patients with diabetes mellitus type 2.

• Co-rrelate vitamin D deficiency (VDD) with Glycemic status of the patients.

• Co-rrealtion of Vitamin D Deficiency [VDD] with other parameters like anthropometry, lipids and blood pressure.

RESEARCH DESIGN AND METHODS

A total 110 diabetes mellitus type 2 patients, (55 males, 55 female) aged 30-70 years were selected by purposive sampling from the heterogeneous population, regularly visiting a diabetic clinic in central Mumbai.

Inclusion criteria

1. Patients with Diabetes Mellitus TYPE 2.with variable duration.
2. Patients with Vitamin D levels reports.

Exclusions criterion

1. Type-1 diabetics
2. Pregnant and Lactating
3. People with hepatic or renal disease

These subjects were examined and questioned in specific format. Data collected and analysed for statistical significance by SSPS 16.4

RESULTS:

The results of the study showed that 75 subjects out of 110 diabetic Type 2 outpatients had lower vitamin (OH)D3 levels(67.2%).Low serum vitamin D3 was found to be non-significantly correlating with Fasting Blood Sugar (P= -.106) and Postprandial Blood Sugar (P= -.106) and also with other parameters like systolic Blood pressure (P=0.083) diastolic Blood Pressure (P= -.082), Obesity markers eg Waist Hip Ratio(P= -.061), Body Mass Index (P=0.102) And with lipid parameters like Triglycerides (P= -.150), total cholesterol (P = -.035) , LDL(P = - 0.35) ,VLDL (P= -0 .154) .However low vitamin D3 was found to be significantly correlating with low HDL levels(P=0.001].

CONCLUSIONS:

The study suggests high prevalence of vitamin insufficiency in Diabetes Mellitus Type-2 outpatients. But there was no significant correlation between low vitamin D3 with blood sugar levels but low vitamin D was shown to be positively correlating with HDL. These finding need to be supported with larger multi centric data and further studies are required to confirm the data. It was also observed that compared to the National standard (97%) for the prevalence of low vitamin D levels, present study had lower prevalence of low vitamin D in diabetes. Thus national wise disease specific multi-centric study for prevalence of vitamin D deficiency with standardized cut off value for Vitamin D has to be made.

PIII-332

B12 Deficiency Is Not Uncommon during Pregnancy in Non-Vegetarian Population Worldwide: A Systematic Review. N. Bawazeet[1], S. Rafnsson[2], C. S. Yajnik[3], P. Saravanan[1]. *[1]University of Warwick, United Kingdom; [2]University of Edinburgh, United Kingdom; [3]KEM Hospital, India.*

In most population, both low and high birth weight is associated with onset of diabetes in later life. This is called "nutrient" and "fuel" mediated teratogenesis, respectively. Studies from India showed children born to mothers with low B12, especially in association with high folate levels, have higher adiposity at birth and higher insulin resistance at six years of age. In addition, mothers with low maternal B12 had higher incidence of gestational diabetes and future type 2 diabetes. The B12 deficiency rate in these studies was high between 40 & 70%, presumably due to vegetarianism. It is not known whether similar phenomenon exists in non-vegetarian population across the world. We did a systematic review to study the prevalence of B12 deficiency during pregnancy.

A comprehensive literature search in six electronic databases for publications on the prevalence of B12 deficiency during pregnancy, screening of reference lists, and citation search was conducted. All databases were searched using a combination of keywords from inception to February 2011. Publications of all studies (longitudinal or cross sectional studies) on normal adult pregnancy were further inspected and studies mentioned the prevalence of B12 deficiency were included.

Thirty-three studies (Americas – 8, Europe & middle east – 4, Africa – 7 & Australasia – 11) have reported the prevalence of B12 deficiency. Studies differed in the population studied, period of blood collection and the cut-off used to define B12 deficiency. Number of studies reporting the B12 deficiency according to the trimesters were: 1st – 7; 2nd – 19 & 3rd – 22. The overall B12 deficiency was between 42-74% in the vegetarian population (six from India and one from Nepal – all 2nd & 3rd trimester) apart from 1 study in Nepal (28.3%; 1st trimester; cut-off 221 pmol/L). In non-vegetarian population, it ranged from 4.5 – 80.9%. The deficiency rates according to the trimesters were: 1st (n=6) – 4.3-44.2%; 2nd (n=17) – 4.5-60% & 3rd(n=15) – 5.7-80%. Studies with rates below 10% used lower cut-off values to define B12 deficiency.

This systematic review showed that B12 deficiency during pregnancy is not uncommon in non-vegetarian population worldwide and can be high similar to the levels seen in vegetarian population. Studies correlating the B12 levels and the metabolic risk of mothers and offspring in non-vegetarian population are urgently warranted.

PIII-333

Low B12 Level Is Associated with Maternal Obesity and Higher Birthweight in Gestational Diabetes. N. Sukumar[1], N. Bawazeer[2], V. Patel[1,2], P. Saravanan[1,2]. *[1]George Eliot Hospital, United Kingdom; [2]University of Warwick, United Kingdom.*

In most populations, both low and high birth weight is associated with onset of type 2 diabetes (T2D) in later life. Studies from India showed low maternal vitamin B12 is related to higher incidence of gestational diabetes mellitus(GDM), higher adiposity & insulin resistance in offspring and future T2D in mothers. It is not known whether similar phenomenon exists in UK population. We aim to study B12 status in pregnancy and investigate its relationship with maternal BMI, incidence of GDM and birth weight in women with and without GDM.

Retrospective study of mothers attending the antenatal diabetes and medical clinics(2005-10) in Warwickshire, UK(mainly Caucasian population). Maternal B12 &folate levels are checked routinely in most women. The inclusion criteria was women without pre-gestational diabetes delivering live, singleton babies.

Of the 270 mothers who met the inclusion criteria, B12 & folate were measured in 209 and in 38 of the 60 women with GDM at median 24 weeks. As expected, GDM mothers were older than non-GDM (33.5 vs 30.5 years, $p<0.001$) and had higher BMI (31.0 vs 26.1, $p<0.001$). The median (IQR) B12 & folate levels were 143.9 (106.3, 194.5)pmol/L and 15.9 (10.0, 25.8) nmol/L respectively.

Women with B12 deficiency(≤150pmol/L) had higher BMI at booking (28.0 vs 25.7, $p=0.013$; controlled for age, smoking, folate and presence of GDM). Women who developed GDM had slightly lower B12 (146.8 vs 172.4pmol/L, $p=0.259$) though this was not statistically significant. Similarly, a non-significant difference was seen in the proportion of B12-deficient women developing GDM (19.1 vs 17%).

GDM mothers with B12 levels below the median had heavier babies (3522.1 vs 3211.1g, $p=0.025$). This difference persisted even after controlling for folate levels, gestation at birth, maternal age and booking BMI (β: -0.39, $p=0.017$). No such difference was seen for non-GDM mothers (3421.8 vs 3345.8g, $p=0.395$).

Our study showed that B12 deficiency is not uncommon in UK Caucasian population; is associated with maternal obesity; possible higher incidence of GDM and macrosomia in GDM. As it is retrospective and observational, causality cannot be ascertained. However, this is the first study to show the possible interaction between B12 levels, GDM, and their additive influence on birth weight. Adequately powered prospective studies are urgently needed on the influence of early pregnancy B12 levels on the risk of GDM and neonatal outcomes.

PIII-334

Maternal Prebiotic Consumption: Beneficial Effects on Offspring Peak Bone Accretion and Structure. Pilar Bueno, Manuel Manzano, María Luisa Jimenez, Ricardo Rueda, José María López-Pedrosa. *R&D, Abbott Nutrition, Granada, Spain.*

Bone mass accrual occurring during the childhood and adolescence is a major determinant of peak bone mass. Maximizing the peak bone mass is currently considered as a key preventative strategy against osteoporosis. Peak bone mass is partly inherited and is strongly influenced by environmental factors, including maternal diet, during uterine and early postnatal life. The aim of this study was to determine the relationship between maternal dietary intake of prebiotic and offspring peak bone mass/architecture in early adulthood female rats.

Offspring from Sprague-Dawley dams fed either standard rodent AIN93-G diet (C) or the same diet supplemented with inulin-type fructans (7.5% total diet) (Syn), during gestation and lactation periods, were maintained on the AIN93-G diet to adulthood. Femur, tibia and vertebrae samples were taken at 16 weeks of age and bone mineral density (BMD), mineral content (BMC) and structure were analyzed by dual energy X-ray absorptiometry (DXA) and computed tomography (μ-QCT), respectively.

Maternal diet supplementation with prebiotic did not affect either BMD/BMC or bone structure of appendicular bones. However, BMD/BMC as well as bone structure of axial bones were significantly influenced by maternal consumption of prebiotic. BMD and BMC of lumbar vertebrae were increased around 20% ($p<0.05$) by prebiotic maternal intake as compared to the control group. In the female offspring, cancellous and cortical vertebrae structural parameters such as BV/TV, trabecular thickness, vBMD and cortical thickness were enhanced between 10 to 15% ($p<0.05$) by prebiotic maternal intake with a parallel reduction in trabecular separation and cortical porosity ($p<0.05$).

Adolescent offspring from mothers fed with inulin-type fructans during gestation and lactation displayed an increased BMD/BMC and improved bone architecture in lumbar spine as compared to the offspring of mothers receiving the standard diet. Based on our study results, in non deficient conditions, prebiotic supplementation might be considered as a plausible nutritional option for optimizing the peak bone and architecture as a strategy to increase bone strength and to prevent bone fragility.

PIII-335

The Association between Early Life Factors and Bone Density and Size in 13 Year-old Urban South African Children. Lisa K. Micklesfield[1,2], Shane A. Norris[1], John M. Pettifor[1]. *[1]Department of Paediatrics, University of the Witwatersrand, South Africa; [2]Department of Human Biology, University of Cape Town, South Africa.*

We have previously shown an association between weight and height at one year, and DXA bone mineral content (BMC) in children aged 10 years. The aim of this study was to determine if early growth is associated with bone density and size measured using peripheral quantitative computed tomography (pQCT) at 13 years of age, and whether or not this relationship is independent of current body composition and size, and puberty.

We examined the association between size (weight, height and BMI) at two years of age, current height, fat and lean mass, and bone parameters in black boys (n=170) and girls (n=150) in the Bone Health subsample of the Birth to Twenty cohort at 13 years of age. We used multiple regression analyses to determine whether the significant relationships between size at two years, and metaphyseal and diaphyseal bone parameters, were dependant or independent of current body composition and size, and pubertal status.

In boys, BMI at two years was significantly associated with metaphyseal (4%) tibia total and trabecular density, before and after adjusting for current height, fat and lean mass. Pubertal development did not make a contribution to the final model for either pQCT measure. Height at two years was significantly associated with diaphyseal (38%) tibia total area but was no longer significant after adjusting for current height, fat and lean mass, or puberty. In the girls, metaphyseal (4%) radius total area was significantly associated with weight at two years. After adjusting for current height, fat and lean mass, and puberty, weight at two years no longer made a contribution to trabecular bone size. Weight at two years was inversely associated with 38% tibia total area in girls, before and after adjusting for current height, fat and lean mass, and then puberty.

Bone density at the trabecular sites was associated with BMI at two years of age independent of current body size and pubertal status in black boys, while height at two years influenced diaphyseal size probably through it effect on current body size. Similarly, in black girls, who were more advanced pubertally, current body size removed the effect of weight at two years on tibial metaphyseal and diaphyseal size. These findings suggest that the pubertal growth in bone mass and size may mask the independent influence of early growth on cortical bone density and size.

PIII-336

Emergence with Aging of Decreased beta Cell Function in Male Offspring of Maternal Rats Fed Normal or Low Protein (LP) Isocaloric Diets. Sumiko Morimoto[1], Lizbeth Calzada[1], Tonantzin C. Sosa[1], Luis A. Reyes[1], Guadalupe L. Rodriguez-Gonzalez[1], Angelica Morales[1], Peter W. Nathanielsz[2], Elena Zambrano[1]. *[1]Reproductive Biology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico DF, Mexico; [2]Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio, TX, USA.*

Reduced dietary protein in pregnancy and/or lactation alters pancreatic islet development predisposing offspring (OFF) to later life diabetes. In addition insulin secretion is impaired with ageing associated with an increased incidence of diabetes and worsening of glucose tolerance. Aim. To investigate in vitro pancreatic function at different ages in OFF exposed to LP diet during development.

Methods. We studied male offspring of rats fed control (C) or LP protein (R) diets in pregnancy (first letter) and/or lactation (second letter) from four groups CC, RR, CR or RC. Serum glucose, insulin and insulin resistance index (IRI) were measured. Pancreatic islets were dispersed and in vitro insulin secretion quantified in low glucose (LG - 5mM) or high glucose (HG - 11mM).

Results. There were no differences between groups at all ages in body weight, glucose and insulin serum values at the same age. Serum insulin and IRI rose with age and were highest at postnatal day (PND) 450 in all groups. At PND 36 insulin secretion (ng/ml) to HG was greatest in RR and RC (CC 0.18 ± 0.01; RR 1.2 ± 0.07; CR 0.9 ± 0.3 and RC 2.3± 0.15, $p<0.05$) and only CC showed a significant increase in insulin secretion to HG compared to LG. By PND 110 all restricted groups responded less to LG (CC 0.36±0.05; RR 0.06±0.02; CR 0.05±0.006; RC 0.17±0.03) but increased secretion to

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HG (CC 0.9±0.2; RR 0.28±0.05; CR 0.39±0.009; RC 0.36±0.02) p<0.05. By PND 450 CC offspring alone increased secretion to HG (0.21± 0.02 vs. 0.39±0.07, p<0.05).
Conclusions. Despite minimal differences in circulating insulin and glucose, reduced maternal protein intake affected islet secretion at all ages. In addition, aging reduced function in all R groups compared with CC by PND 110 and further by PND 450 with the most marked effect in RC. We conclude that LP during development alters the trajectory of aging of pancreatic insulin secretion, and confirm our hypothesis that beta-cell responses are blunted in offspring exposed to LP diet and that reduced function with aging is influenced by the developmental stage in which nutrition is decreased.

PIII-337

Developmental Influences on Skeletal Muscle Gene Expression in Older Men: Findings from the Hertfordshire Sarcopenia Study.
Harnish P. Patel[1,2], Nasser Al-Shanti[3], Sheila J. Barton[1], Claire E. Stewart[3], Miranda D. Grounds[4], Ross L. Tellam[5], Cyrus Cooper[1], Avan A. Sayer[1,2].
[1]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom; [2]Academic Geriatric Medicine, University of Southampton, United Kingdom; [3]Institute for Biomedical Research into Human Movement and Health, Manchester Metropolitan University, United Kingdom; [4]School of Anatomy and Human Biology, University of Western Australia, Australia; [5]CSIRO Livestock Industries, Brisbane, Australia.
Introduction
Consistent relationships between lower birthweight and reduced adult grip strength, a key component of sarcopenia have been demonstrated. Furthermore, a recent study has shown that low birthweight is associated with a reduced muscle fibre score in older men. However, the underlying molecular mechanisms are not known. Anabolic and catabolic pathways are important regulators of adult muscle but whether they are associated with growth in early life has not been previously explored. Our aim was to investigate the relationship between small size at birth and gene expression of key intracellular signalling molecules within skeletal muscle late in life.
Muscle biopsies of the vastus lateralis were obtained from men aged 68-76 years with lower (≤ 2.64 kg, n=7) and higher (≥ 4.54 kg, n=12) birthweight. TaqMan PCR arrays were used to determine the expression profiles of 44 genes implicated in the regulation of skeletal muscle and were grouped into functions that were predominantly anabolic or catabolic. Fold changes in gene expression were determined in the lower, relative to the higher birthweight group. Median levels of gene expression were compared using Mann-Whitney U tests.
Genes in the anabolic group: MAPK8, IGFBP3, and IL15, showed fold changes of 4.90, 1.99 and 0.51, respectively (Mann-Whitney [M-W] p=0.144, p=0.031, p=0.042, respectively). The catabolic gene lipoprotein lipase (LPL) showed a fold change of 2.07 (M-W p= 0.073). There were no other significant fold changes in gene expression.
This is the first study of skeletal muscle gene expression in older men with historical records of birthweight. The results suggest that skeletal muscle of older men with lower birthweight display mainly anabolic expression profiles that could be consistent with compensatory mechanisms that have arisen as a consequence of relatively fewer myofibres. Replication studies in older women as well as other groups of men are now needed.

PIII-338

Past History of Mental Disorders Associates with Longer Peripheral Blood Cell Telomeres in Elderly Adults: The Helsinki Birth Cohort Study (HBCS).
Katri Savolainen[1], Katri Räikkönen[1], Laura Kananen[2,3], Eero Kajantie[4,5], Iiris Hovatta[2,3,6], Marius Lahti[1], Johan G. Eriksson[4,7,8,9,10].
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Mental disorders have previously been linked with accelerated telomere length (TL) shortening and early mortality. It remains unclear if accelerated TL shortening characterize mental disorders patients who have survived till older age.
The participants were 61.5 (SD=2.9,Range=56.6–69.8) year-old women (n=1051) and men (n=905) from the HBCS. TL from peripheral blood cells was measured using real-time quantitative PCR method. Individuals with mental disorders severe enough to warrant hospitalization (n=116) were identified from Finnish Hospital Discharge Register. The average time from last hospitalization to measurement of TL was 12.9 (SD=8.9,Range=0.3-31.3) years.
Participants hospitalized for any mental or substance use disorders had longer TL than non-hospitalized controls (age and sex adjusted p-values<0.042). Adjustments for smoking, alcohol consumption, exercise, highest education attainment, physician diagnosed coronary heart disease or purchases of psychiatric, diabetes or hypertension medication did not change the result. Mental disorder hospitalization 0.3-10 (mean=5.3,SD=2.90,Range=0.3-9.99) years prior to TL measurement had the strongest positive effect on TL (p=0.023). A combination of low birth weight (<2500g) and mental disorders was also connected to longer TL (p=0.018).
These results contradict previous evidence showing that mental disorders and shorter TL are associated. Our findings suggest that a past history of mental disorders associates with longer TL in a population who has survived into older adulthood.

PIII-339

Grip Strength at Age 58 after Prenatal Exposure to the Dutch Famine.
Marjolein V. Veenendaal[1], Susanne R. de Rooij[1], Rebecca C. Painter[2], Sian M. Robinson[3], Clive Osmond[3], Avan Aihie Sayer[3], Tessa J. Roseboom[1].
[1]Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, University of Amsterdam, Netherlands; [2]Department of Obstetrics and Gynaecology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands; [3]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom.
Grip strength is an important marker of current and future health. Small size at birth is associated with reduced grip strength and poor health. It is thought that prenatal undernutrition may affect grip strength in later life.
The aim of this study is to investigate the relationship of prenatal undernutrition and grip strength the Dutch famine birth cohort.
We assessed hand grip strength in 334 men and 364 women at age 58, born as term singletons around the time of the 1944-45 Dutch famine. We compared grip strength among men and women who had been exposed to famine during different periods of gestation to that of those who had not been exposed prenatally.
Men exposed to famine in early gestation appeared to have greater grip strength, unadjusted 4.15 kg (95%CI 0.96 to 7.33), compared to unexposed men. However this group also had non-significantly higher birth weight, adult weight and height, and after adjustment for adult size and timing of participation in the study, the association was no longer significant (2.91 kg (95%CI -0.18 to 5.99)). In women, there was no association between prenatal exposure to famine and adult grip strength. A one kilogram increase

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in birth weight was associated with an increase of 2.84 kg (95%CI 0.95 to 4.72) in grip strength in men and 1.46 kg (95%CI 0.08 to 2.83) in women, adjustment for adult body size explained this relationship.
Prenatal exposure to undernutrition was not significantly associated with adult grip strength in this cohort. Consistent with previous studies, there was a relationship between small size at birth and lower adult grip strength which was largely explained by associations between size at birth and adult body size.

PIII-340

Growth during Infancy and Postprandial Appetite Regulatory Hormone Responses in Later Life. Mia-Maria Perälä[1], Liisa M. Valsta[2,3], Eero Kajantie[1,4], Johan G. Eriksson[1,5]. *[1]Department of Chronic Disease Prevention, National Institute for Health and Welfare, Finland; [2]Department of Lifestyle and Participation, National Institute for Health and Welfare, Finland; [3]Data Collection and Exposure, European Food Safety Authority, Italy; [4]Hospital for Children and Adolescents, Helsinki University Central Hospital, Finland; [5]Department of General Practice and Primary Health Care, University of Helsinki, Finland.*
Slow growth during infancy is associated with increased risk of type 2 diabetes and cardiovascular disease in later life. While obesity is closely linked with these disorders, adult obesity seems to be related with rapid growth during infancy. It has been suggested that appetite regulatory hormones may be programmed in early life, however, data to support this are lacking. Our aim was to examine the impact of growth during infancy on postprandial responses of two high-protein content meals: a calcium caseinate meal (Casein-meal) and a whey protein isolate meal (Whey-meal).
We recruited 24 overweight 65-75 year-old subjects, 12 with slow growth during infancy (SGI-group) and 12 with normal early growth (CON-group). The study meals contained the same amount of energy, carbohydrate, protein and fat. Both meals were consumed once in a random order. Plasma glucose, insulin, triglycerides (TG), free fatty acids (FFA), glucagon, GIP, and appetite regulatory hormone ghrelin, PYY, and GLP-1 were measured in fasting state and over a 4-h period after both study meals. The incremental areas under the response curves were calculated.
Fasting concentrations did not differ significantly between the groups. Postprandial responses of insulin and PYY were higher for the SGI-group both after the Casein-meal (P=0.017 and p=0.025, respectively) and the Whey-meal (P=0.057 and p=0.046). The TG responses were higher for the SGI-group after the Whey-meal (P=0.05) and a similar trend was seen after the Casein-meal (P=0.16). Glucose, FFA, glucagon, ghrelin, GIP, and GLP-1 responses did not differ between the groups. The Whey-meal resulted in significantly higher insulin (P<0.001), GIP (P=0.007) and glucagon (P=0.038) responses compared with the Casein-meal. No differences were seen between study meals in TG, FFA, glucose, ghrelin, PYY nor GLP-1 responses within the groups.
Early growth predicts higher postprandial insulin and TG responses. Growth during infancy may also have a role in programming appetite regulatory hormone secretion in later life.

PIII-341

SIRT1-Mediated Epigenetic Regulation Contributes to Reduced Hypothalamic Neural Stem Cells (NSC) Proliferation and Differentiation in LBW Newborns. Mina Desai, Tie Li, Michael G. Ross. *Obstetrics & Gynecology, David Geffen School of Medicine at UCLA and Los Angeles Biomedical Research Institute, CA, USA.*
Human and animal low birth weight (LBW) newborns have increased risk of adult obesity. Using a rat model of maternal food restriction, we have demonstrated that LBW newborns are hyperphagic and have reduced hypothalamic anorexigenic cellular signaling responses, suggesting altered neurodevelopment. Further, the neural stem cells (NSCs) from LBW newborns exhibit programmed reduced proliferation and differentiation. Notably, this process is epigenetically regulated by histone modification and DNA methylation. As such, we have further shown that the hypothalamic nutrient sensor, SIRT1 (histone deacetylase) is upregulated whereas DNA methyltransferase (DNMT1) is downregulated. This occurs in conjunction with reduced Notch1/Hes1 (promotes NSC proliferation and inhibits differentiation). In view of this, we confirmed the role of SIRT1 and DNMT1 in NPC proliferation and determined the interaction with Hes1 promoter.
Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to term (LBW). Hypothalamic NSCs were cultured in complete medium from one day old LBW and Control newborns. Chromatin was harvested for ChIP (anti-Sirt1). Pulled-down chromatin was used as template for PCR primer specific to Hes1. Further, NSCs were transfected with control or SIRT1/DNMT1 siRNA. Cell proliferation (MTT assay), and protein expression of markers of NSC (nestin), proliferation (Hes1), neurons (Tuj1) and astrocyte (GFAP) were determined.
As expected, siRNA reduced SIRT1 and DNMT1 expression. SIRT1 silencing promoted NPC proliferation with increased nestin and Hes1 protein expression, suggesting a role of SIRT1 in Hes1-mediated NPC proliferation. In contrast, DNMT1 silencing inhibited NPC proliferation and Tuj1 expression and increased GFAP, suggesting that increased astrogenesis may be mediated via DNMT1 methylation of GFAP. Furthermore, SIRT1 pull down of Hes1 was significantly higher in LBW, suggesting increased SIRT1/Hes1 promoter binding and hence increased deacetylation of Hes1, leading to suppression of Hes1.
In LBW newborns, reduced neurogenesis is epigenetically mediated via histone (SIRT1) and DNA (DNMT1) modifications. We speculate that increased SIRT1 which reduces both Hes1 and DNMT1 levels, induces premature differentiation and reduces NSC pool in LBW offspring.

PIII-342

Maternal Obesity Is Associated with Altered Appetite and Hypothalamic ObR, MC4R, and FTO Expression in Mice Independently of Postnatal Diet. Anne-Maj Samuelsson[1], Sylvain Sebert[5], Shikta Das[2], Marika Kaakinen[3], Ulla Sovio[4], Jaana Laitinen[6], Anneli Pouta[7], Lucilla Poston[1], Marjo-Riitta Jarvelin[2,3,7], Paul D. Taylor[1]. *[1]Division of Women's Health, Kings College, London, United Kingdom; [2]Department of Epidemiology & Biostatistics, Imperial College London, London, United Kingdom; [3]Institute of Health Sciences & Biocenter, Oulu, Finland; [4]London School of Hygiene and Tropical Medicine, United Kingdom; [5]Human Development, University of Nottingham, United Kingdom; [6]Finnish Institute of Occupational Health, Finland; [7]National Institute of Health and Welfare, Finland.*
We have previously reported that maternal obesity in rodents has a significant impact on obesity and hyperphagia in adult offspring. This study investigates the interaction between maternal obesity and a postnatal obesogenic environment on the development of obesity and altered appetite/satiety control in the hypothalamus.
C57BL/6J female mice were fed either a standard chow (7% simple sugar, 3% fat) or a highly palatable obesogenic diet (33% simple sugar,16% fat) 6 wks prior to mating and throughout gestation and lactation. Offspring were then weaned onto chow or high calorie 'Western' diet (HC, 10% simple sugar, 20% fat). At 3 M of age, appetite was recorded and body and organ weight measured. Micro-dissection of the hypothalamus was performed and ObR, MC4R, and FTO mRNA expression measured using Real-Time PCR.
Male and female offspring of obese dams (OffOb) reared on chow were heavier and fatter than control offspring fed on chow (OffCon). OffOb maintained on the HC postnatal diet demonstrated significantly greater adiposity than OffOb weaned to control diet. At 3 M of age, calorific intake/body weight (CI/BW) was higher in OffOb *v* OffCon when fed control chow ([kcal/g] male OffOb, 0.79±0.06 v OffCon, 0.62±0.03, p<0.01; female OffOb, 0.75±0.04 v OffCon, 0.46±0.05, p<0.001). Hypothalamic ObR mRNA expression was increased in male OffOb and decreased in female OffOb compared to controls on standard chow. OffOb demonstrated increased hypothalamic FTO and MC4R mRNA with no additive effect of HC postweaning diet.
Post-natal hypercalorific diet had an exaggerated effect on weight gain and adiposity in Offob. Whilst postnatal hypercalorific diet elevated appetite in OffCon there was no additive effect on CI/BW in OffOb. Maternal obesity was associated with increased FTO and MC4R expression in the hypothalamus independent of a postnatal obesogenic environment.

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PIII-343

What the Maternal Heart Tells the Fetal Brain. Curt A. Sandman[1], Elysia P. Davis[1,2], Christine Cordova[1], Arron Kemp[1], Laura M. Glynn[1,3]. *[1]PSYCHIATRY AND HUMAN BEHAVIOR, University of California, Irvine, CA, USA; [2]PEDIATRICS, University of California, Irvine, CA, USA; [3]CREAN SCHOOL OF HEALTH AND LIFE SCIENCES, Chapman University, CA, USA.*

The human fetus is exposed to 26,000,000 maternal heart beats throughout gestation. This 95db signal is the predominant source of continuous auditory and pressure communication between the mother and her fetus. There is strong evidence that the fetus responds to maternal heart rate variations, including a fetal evoked brain potential to maternal ventricular contractions. The primary aim of this study is to examine the developmental consequences for the human fetus, of exposure to stable versus fragmented maternal heart beat variations.

Maternal heart beat patterns at 25, 30 and 35 weeks gestation were assessed using linear and non-linear time series analysis. Short- and long-term fragmentation and chaotic pattern-sequences were identified. Emotional and cognitive consequences of fetal exposure to these patterns were assessed in children from three to 24 months of age.

Fetal exposure to fragmented or chaotic maternal heart beats determined by autocorrelations and non-linear pattern analysis resulted in temperamental outcomes persisting for at least 12 months postpartum. Exposure to unstable or fragmented maternal heart beats was associated with negative affectivity and with surgency/extraversion. These effects appeared to be restricted to affective behavior and not cognition. There were several findings related to the timing of exposure during gestation.

Recent findings from our group indicated that the pattern of maternal care in an animal model, and not the frequency or duration of care, produced profound changes in offspring behavior and in the structure of the nervous system. The current findings indicate that fetal exposure to predictable or stable maternal information may program temperamental patterns. To our knowledge these are the first findings that have demonstrated the influence of fetal exposure to maternal heart beat patterns on infant development. These findings suggest that the fetal brain may be entrained by the continuous and dominating influence of the maternal heart.

PIII-344

Blockade of Type 1 Cannabinoid Receptors Simultaneous to Nociceptive Stress during Early Lactation, Generates Leptin Resistance and Metabolic Syndrome in Adult Mice. Paulo Silva, Valeska Castillo, Carolina Aguirre, Liza Fonseca, Ana Ronco, Miguel Llanos. *Instituto de Nutrición y Tecnología de los Alimentos. Universidad de Chile, Santiago, Chile.*

Nociceptive stress (NS) during lactation induces metabolic disturbances and overweight in adult mice. We have recently suggested that overactive type 1 cannabinoid receptors (CB_1R) present in tissues involved in energy homeostasis may be part of the mechanism linked to these alterations. If this overactivity is triggered during lactation, it may be possible that blocking CB_1R during this period could prevent NS late effects. The aim of this study was to evaluate long term metabolic effects of NS during early lactation simultaneous to treatment with a CB_1R antagonist.

Male mice pups were randomly distributed for maternal cross-fostering. In the early lactation (day 1-10), mice were stressed (NS) with a subcutaneous injection of saline in the back and immediately treated with an oral dose of the CB_1R antagonist AM-251 (1 ug/g body weight). This was the main AM-NS group. Appropriate control groups were designed for these treatments: NS vehicle-treated (NS-V), no NS AM-251-treated (C-AM) and no NS vehicle-treated (C-V). Food intake and body weights were measured every 10 days. In 120-140 days old mice, glucose tolerance, insulin and leptin sensitivity tests were performed. Plasmatic metabolic markers and blood pressure (BP) values were also determined.

Unexpectedly, from day 40 to 140, AM-NS group showed significant overweight and increased food intake over C-V ($p<0.05$; Kruskall-Wallis and Mann-Whitney U test), although similar to NS-V groups. All groups had significant increases in epididymal fat pads *v/s* C-V. AM-NS group showed significant highest values of systolic arterial BP and plasma levels of corticosterone, glucose, triglycerides, cholesterol and leptin over C-V ($p<0.05$). Interestingly, only AM-NS mice developed a marked insulin and leptin resistance.

These results show that blocking CB_1R simultaneous to NS cause long term alterations in glucose homeostasis with other physiological disturbances consistent with the metabolic syndrome. Since CB_1R are necessary for habituation to stress, the simultaneous NS and transient loss of CB_1R activity may act synergistically to exert a reprogramming and/or developmental compensation of CB_1R expression and/or CB_1R-related actions in different tissues leading to metabolic disease in adulthood. Funded by FONDECYT-CHILE Grant 1100145

PIII-345

Programming Effects of Neonatal Ghrelin on Appetite-Related Hypothalamic Circuits. Sophie M. Steculorum[1,2], Sebastien G. Bouret[1,2]. *[1]The Saban Research Institute, University of Southern California, Los Angeles, CA, USA; [2]INSERM U837, University of Lille 2, Lille, France.*

Ghrelin is a pleiotropic hormone originally described to promote food intake, which effects are likely mediated by the arcuate nucleus of the hypothalamus (ARH). In addition to its orexigenic effects, ghrelin has been shown to be involved in perinatal development. Recent data from our laboratory have indicated that ghrelin is able to act within the hypothalamus during the time frame of hypothalamic circuit development, suggesting that ghrelin maybe involved in the formation of these neural pathways.

In the present study, we explored the consequences of blocking ghrelin signaling specifically during early postnatal life on metabolic regulation and hypothalamic development.

The results indicate that administration of a ghrelin-neutralizing agent from postnatal day (P) 4 to P21 results in a significant increase in pre- and post-weaning body weight curves, when compared to control animals. In addition, animals treated with anti-ghrelin neonatally were hyperphagic, hyperglycemic, and displayed increased adiposity during adulthood. Interestingly, these metabolic alterations were associated with changes in the development of neuronal projections from the arcuate nucleus of the hypothalamus. Neonates treated with the anti-ghrelin have an enhanced development of ARH projections and these alterations persist into adulthood. In vivo experiments using ARH explants further revealed that ghrelin acts directly on ARH neurons to blunt axon growth.

Collectively, these data indicate that ghrelin is required for normal development of hypothalamic circuits involved in the control of body weight and glucose homeostasis and that disruption of ghrelin signaling during critical periods of development compromises metabolic regulation throughout life.

PIII-346

The Effects of Maternal High Fat Feeding on Body Fat Mass and Susceptibility to Diet Induced Obesity Can Be Reversed by Interventions during the Suckling Period. Mini A. Vithayathil[1], Zhi Y. Ong[1,2], Beverly S. Muhlhausler[1,2]. *[1]FOODplus Research Centre, School of Agriculture, Food and Wine, The University of Adelaide, South Australia, Australia; [2]Sansom Institute for Health Research, Unversity of South Australia, South Australia, Australia.*

There is growing evidence that perinatal exposure to excessive nutrient supply contributes to an increased risk of obesity in the offspring. However, it is unclear whether and to what extent the effects of prenatal exposure to an excess nutrient supply can be reversed. The present study investigated the hypothesis that the effect of maternal high-fat feeding on fat mass and susceptibility to diet induced obesity in the offspring would be prevented by cross-fostering the pups onto a Control dam at birth.

Albino Wistar dams were fed either standard rat chow (Control, n=11) or high-fat cafeteria diet (HF, n=10) for four weeks before pregnancy and during pregnancy and lactation. All offspring were cross-fostered onto a dam from either from the same or different treatment group and were fed standard rat chow after weaning (3 weeks of age). At three months of age, all offspring were provided with free access to a high-fat cafeteria diet. Body weight and food intake was determined weekly and body fat mass determined at three week, six weeks and four months of age.

HF pups suckled by another HF mother (HF-HF) had a higher fat mass at weaning compared to Control offspring (HF-HF, $0.52 \pm 0.007\%$; C-C, $0.35 \pm 0.003\%$, $p<0.0001$), and this effect was reversed by cross-fostering the HF offspring onto a Control dam (HF-C, $0.25 \pm 0.004\%$ vs $0.35 \pm 0.003\%$, p=ns). There were no differences between the groups in fat mass at six weeks

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of age. Pups exposed to the HF diet during the suckling period, but not HF offspring cross-fostered onto a Control dam at birth, exhibited an increased preference for fat and sugar intake at three months of age (P<0.001), and an increased percentage body fat after four weeks on a HF diet (P<0.001).
Our findings demonstrate that the effects of exposure to a HF diet during fetal life can be reversed by removing the HF stimulus during the suckling period. These data highlight the importance of identifying the critical windows for the programming of body fat mass, and the potential for reversing the the effects of prenatal exposure to HF diets by interventions applied after birth.

PIII-347

Maternal Western Style Diet Programs the Development of Obesity and Fatty Liver in Mice. Maurien Pruis, Agnes Lendvai, Mathijs V. Zwier, Albert K. Groen, Torsten Plosch. *Pediatrics, University Medical Center Groningen, Groningen, Netherlands.*
Obesity is associated with increased risk of various complications of pregnancy. Adding to this, animal models have shown indications that obesity in the mother also plays a direct role in transmission of an obesogenic and diabetogenic trait from generation to generation. We here aimed to identify the underlying mechanism. We investigated the short and long term effects of maternal western style diet on adult offspring.
Female C57BL/6 mice were fed a semi-synthetic low fat diet (LF, 10 kcal% fat and 18 mg/kg cholesterol) or a semi-synthetic western style diet (WS, 45 kcal% fat and 196,5 mg/kg cholesterol) before and during gestation and lactation. From weaning on offspring received the LF diet or WS diet, resulting in four groups: LF/LF, LF/WS, WS/WS and WS/LF (diet of the mother/offspring).
Surprisingly, there were no alterations in body weight and glucose concentration between dams fed with WS or LF diet before and during gestation and lactation. However, at the end of the lactation period dams on the WS diet showed a different plasma and liver lipid profile. In male offspring prenatal exposure to WS diet led to a higher body weight over time in both postnatal diet groups. Plasma lipids were not affected by intrauterine exposure to a WS diet or by adult WS diet feeding. In contrast, liver size increased by 1.9 fold respectively in WS/WS offspring (p<0,001), also associated with a rise in liver lipid concentrations. This increase in liver size was not seen in the WS/LF or LF/WS diet offspring. Histological markers showed differences among the post weaning dietary groups (WS versus LF), and this effect was markedly exaggerated when offspring had been also exposed to a WS diet during early developmental stages.
Our data provide evidence that exposure to a WS diet in early developmental stages predisposes offspring to obesity and primes susceptibility to develop liver hypertrophy and hepatic steatosis.
This research was performed within the framework of CTMM, the Center for Translational Molecular Medicine www.ctmm.nl, project PREDICCt grant 01C-104, and supported by the Netherlands Heart Foundation, Dutch Diabetes Research Foundation and Dutch Kidney Foundation.

PIII-348

First Trimester Weight Loss Associated with Increased BMI at Age 5: The Amsterdam Born Children and Their Development Study. Tessa Roseboom[1], Rebecca Painter[1], Marcel Van der Wal[2], Joris Van der Post[1], Tanja Vrijkotte[3]. *[1]Obstetrics and Gynaecology, Academic Medical Center Amsterdam, Netherlands; [2]Youth, Public Health Service Amsterdam, Netherlands; [3]Department of Public Health, Academic Medical Center Amsterdam, Netherlands.*
Prenatal undernutrition has lasting negative consequences for health. We here investigate effects of weight loss in early pregnancy on BMI and metabolic profile of five year old children.
The Amsterdam Born Children and their Development study (ABCD-study) is a large multi-ethnic cohort study that was set up in 2003 to include all pregnant women in Amsterdam in that year. Detailed information on life style, psychological conditions, nutrition and socio-demographic background was recorded and these women as well as their children are followed. 3263 children were examined at the age of five years. Using linear regression analyses we compared the BMI and fasting glucose and lipid levels of children whose mothers lost >5kg in the first trimester with that of those whose mothers did not lose > 5kg in the first trimester of pregnancy.
154 mothers of the 3263 children that were examined at age five had indicated during pregnancy that they lost more than 5 kg in the first trimester. These women were younger, more obese, and more often of non-Dutch origins. Birth weights of their children did not differ. At age 5, these children were taller and fatter (BMI was 0.73 units higher, 95% CI 0.49 to 0.96). After adjustment for maternal characteristics (pre-pregnancy BMI, parity, age, ethnicity) and infants sex this difference was 0.34 (95% CI 0.11 to 0.57). The glucose and lipid levels of these children were not different.
Children whose mothers lose weight in early pregnancy are more obese at the age of 5. This is only partly explained by the fact that their mothers were more obese before pregnancy. This suggests that weight loss in early pregnancy may have negative long term consequences for her offspring's health.

PIII-349

A Study To Determine the Influence of a Post-Natal Hypercalorific Diet on the Developmental Programming of Obesity and Hypertension in Offspring of Obese Mice. Anne-Maj Samuelsson, Katherine L. Redington, Esna Uppal, Lucilla Poston, Paul D. Taylor. *Division of Women's Health, King's College London and King's Health Partners, United Kingdom.*
Maternal obesity may have long term consequences for the developing child. We have previously observed hypertension of sympathetic origin in the offspring of diet induced obese mice. In this study we have determined the influence of a high energy diet fed post-weaning to the offspring of obese and control dams.
C57BL/6J female mice were fed either a standard chow (7% simple sugars,3% fat) or a highly palatable obesogenic diet (33% simple sugars, 16% fat) six weeks prior to mating and throughout gestation and lactation. Offspring were then weaned onto standard chow or high calorific diet (HC, 10% simple sugars, 24% fat). At three months of age, blood pressure and pressor response to acute restraint stress were recorded by radiotelemetry. Renal norephinephrine was measured by ELISA.
Male and female offspring of obese dams (OffOb) reared on chow were heavier and fatter than control offspring fed on standard chow (OffCon). OffOb maintained on the HC postnatal diet demonstrated significantly greater weight gain and adiposity than OffCon weaned to the same diet (fat pad mass [mg], OffOb-HC, 155.6±35.7 v OffCon-HC, 95.4±19.6, n=6, p<0.05). At three months of age, systolic blood pressure (SBP) was higher in OffOb v OffCon when both were fed chow (SBP [mmHg] OffOb, 142.3 ± 1.1 v OffCon, 121.7 ± 1.7, n=6, p<0.001). The HC postweaning diet was associated with higher SBP in three month old OffCon v chow fed OffCon (SBP [mmHg], OffCon-HC 148.9 ± 2.6, v OffCon-chow, 121.7 ± 1.7, n=6, p<0.001). However there was no additive effect of the postweaning HC diet in OffOb. Renal norephinephrine content was increased to a similar degree by maternal obesity and postweaning HC diet.
Post-natal hypercalorific diet had an exaggerated effect on weight gain and adiposity in OffOb. Whilst post-natal hypercalorific diet elevated systolic blood pressure in OffCon there was no additive effect on blood pressure in the hypertensive OffOb mice. This suggests that the hypertension in the offspring of obese dams may arise through similar mechanism as the diet induced hypertension observed in the control mice. The increase in renal norephinephrine content suggests underlying sympathetic origin to the hypertension in both conditions.
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PIII-350

Consequences of Obesity during Pregnancy on Fetal Liver Fat Accumulation. Rita S. Strakovsky[1], Yuan-Xiang Pan[1]. *[1]Division of Nutritional Sciences, University of Illinois, Urbana-Champaign, Urbana, IL, USA; [2]Department of Food Science and Human Nutrition, University of Illinois, Urbana-Champaign, Urbana, IL, USA.*
Background and aims: The rates of gestational obesity have been increasing at the same rapid rate as the obesity epidemic in the general population. These statistics are alarming not only because obese pregnancies are accompanied by various birth complications, but also because human and animal studies have shown that obese pregnancy programs offspring for a variety of adult onset diseases. The present study examined the mechanisms behind the effect of maternal obesity on fetal liver lipid accumulation and metabolism.

Methods: Pregnant Obese Prone (OP) and Obese Resistant (OR) rat strains were fed a control diet throughout gestation. Fetal livers were collected on gestational d21 for analysis of physiological observations and gene expression. Rat hepatocyte cells were also treated with NEFA to exam the effects on fat accumulation.
Results: Maternal plasma NEFA and TG were elevated in OP dams, and offspring of OP dams were smaller than OR. Livers of OP offspring had increased TG content and lipid accumulation when compared to offspring of OR dams. Additionally, hepatic *Dkk1* mRNA content was significantly decreased in OP livers when compared to OR, and treating rat hepatocyte cells with NEFA showed that *Dkk1* mRNA was also decreased in NEFA-treated cells. Analysis of the *Dkk1* promoter in fetal livers showed a pattern of histone modifications associated with decreased gene transcription in OP offspring, which supports our gene expression data.
Conclusions: Our results demonstrate that offspring hepatic *Dkk1* is epigenetically regulated via histone modification in the current model of gestational obesity, and future studies will be needed to determine whether these changes contribute to excessive hepatic lipid accumulation in offspring of obese dams.

PIII-351

Which Early Life Factors Underlie Overweight Resilience in Childhood? Lenie van Rossem[1], Alet H. Wijga[2], Marjan Kerkhof[3], Dirkje S. Postma[4], Henriette A. Smit[1]. *[1]Julius Center for Health Science and Primary Care, University Medical Center Utrecht, Netherlands; [2]National Institute for Public Health and the Environment, Netherlands; [3]Department of Epidemiology, University Medical Center Groningen, Netherlands; [4]Department of Pulmonology, University Medical Center Groningen, Netherlands.*
Overweight in early life is a strong risk factor for the development of overweight at school age. However, a considerable proportion of children with early life overweight, attain a normal weight nevertheless. In this study, we investigated factors for 'overweight resilience' by comparing pre- and perinatal characteristics of those children who went on to have overweight (persistent overweight), with those who lost their overweight before the age of 11 years (declining overweight).
We used data from 3550 children participating in an ongoing birth cohort, the PIAMA study. Body mass index was calculated as weight/height^2 at the ages of three months, yearly between the ages of 1-8 years, and at 11 years. A BMI $\geq 90^{th}$ percentile for age was defined as overweight. Latent class growth modelling was used to distinguish overweight trajectories. The 436 children who were overweight at birth were included in the analysis (12.3% of the total study population). Of those, 303 children lost their overweight (8.5% of total population) and 133 children kept their overweight up to the age 11 years (3.8% of total population). We considered parental educational level, parental overweight, maternal smoking during pregnancy, gender, child's ethnicity, birth weight, and breastfeeding as potential factors for 'resilience of overweight'. Persistent overweight was the reference category and declined overweight was the outcome variable to determine associations with logistic regression analyses.
Relative to children who were persistent overweight, children who declined in probability of being overweight were less likely to have an overweight mother (OR: 0.59, 95% CI 0.45-0.75), or an overweight father (OR: 0.72, 95% CI 0.55-0.94). Mother's educational level, maternal smoking during pregnancy, child's ethnicity, birth weight, and breastfeeding duration were not associated.
Overweight children from parents without overweight are more likely to loose their overweight during their schoolyears. Whether this is determined by genetic characteristics or parent's lifestyle should be further investigated.

PIII-352

Identification of Post-Transcriptionally Regulated Genes in Adipose Tissue of Maternal Protein Restricted Offspring Using a Genome-Wide Polysome Profiling Microarray Approach. Matthew Warner[1], Denise Fernandez-Twinn[1], Giles Yeo[1], David McCullough[2], Anne Willis[2], Kenneth Siddle[1], Martin Bushell[2], Susan Ozanne[1]. *[1]Metabolic Research Laboratories, Institute of Metabolic Science, University of Cambridge, Cambridge, United Kingdom; [2]Medical Research Council Toxicology Unit, University of Leicester, Leicester, United Kingdom.*
Epidemiological studies have revealed that low birthweight is associated with increased risk of type 2 diabetes. The early environment plays an important role in mediating this relationship but the underlying molecular mechanisms remain poorly defined. Studies in low birth weight humans and maternal protein restricted rodent offspring showed reductions in key insulin signaling proteins without reductions in the corresponding mRNAs, implicating post-transcriptional mechanisms. Here we adopt a genome-wide approach to identify transcripts programmed at the level of translation.
Rats were fed a control (C) or low protein (LP) diet during pregnancy and lactation. Offspring were weaned onto standard lab chow and at 12 weeks of age adipose tissue collected. Actively translating (polysomal) mRNAs were separated from non-translating (sub-polysomal) transcripts and analysed using Affymetrix Gene ST 1.0 arrays and Genespring GX software. To increase stringency, data was normalized using both RMA and PLIER algorithms and transcripts showing a >1.2 fold change ($p<0.05$; LP vs. C offspring) were subjected to Ingenuity pathway analysis.
159 transcripts were differentially regulated post-transcriptionally (72 decreased and 87 increased). Ingenuity pathway analysis revealed significant enrichment of genes involved in tissue inflammatory responses and cell cycle/death regulatory pathways. Post-transcriptional gene regulation was confirmed by qRT-PCR analyses of sub-polysome and polysome RNA samples for a selection of genes including *lep ($p<0.05$)*, *hspb1 ($p<0.01$)*, *fdx1l ($p<0.05$)* and *slc7a10 ($p<0.05$)*. None of these genes displayed differential expression in total RNA extracts providing further evidence for their post-transcriptional regulation by maternal diet.
We have successfully applied polysome profiling analysis to adipose tissue isolated from maternal protein restricted rodent offspring and identified differentially regulated mRNAs at the post-transcriptional level. This study demonstrates that post-transcriptional gene regulatory events may provide a key mechanism by which nutrition during early life influences future disease risk.

PIII-353

Muscarinic Receptors Subtypes M2/3 Function Is Deregulated by Early Maternal Protein Restriction in Pancreatic Islets from Adult Rats. Júlio C. de Oliveira, Luiz F. Barella, Renato C.S. Branco, Rosiane A Miranda, Luiz A. Bataglini, Rosana Torrezan, Clarice Gravena, Paulo C.F. Mathias. *Department of Cell Biology and Genetics, State University of Maringá, Paraná, Brazil.*
It has been suggested that maternal undernourishment is associated with functional changes in pancreatic islets, insulin secretion and glucose metabolism. Acetylcholine release by neural ends binds to muscarinic receptors (mAChR) which potentiate glucose-induced insulin secretion. There are five subtypes of mAChR; it has some evidence that mAChR odd numbers are insulinotropic while even ones are insulinostatic. We investigated the effect of maternal protein restriction on mAChR function during glucose tolerance test and insulin secretion in the pancreatic beta cell.
Dams received a poor protein diet (4%) during initial 2/3 of lactation (LP), while, control-dams (NP) received normal protein diet (23%), after that both offspring ate normal diet until 90-day-old. In vivo and in vitro the mAChR function was studied. Islets were isolated and incubated/60min with acetylcholine, metoctramine or 4-DAMP, and in another butch of rats the same drugs were injected, 5min before the intravenous glucose tolerance test (ivGTT).
Acetylcholine caused 15.7% glycemic reduction in NP rats; whereas, the same treatment on LP animals provoked 37% glycemic decrease, $p<0.001$. Metoctramine induced decline of plasma glucose in both animal groups; however, the decrease was deeper to LP rats (30.5%) than NP ones (20.1%), $p<0.001$. On the other hand the 4-DAMP elevated the plasma glucose only

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in NP rats (13.8%, p<0.001) without changing it in LP ones. In vitro, the glucose-induced insulin secretion of LP-group was lower than NP in all glucose concentration. Insulinotropic effect of ACh was higher in LP rats (91.3%) than NP ones (74.8%). In the both groups, metoctramine-treatment did not change insulin secretion, although 4-DAMP-treatment reduced it in (LP, 70.3% *vs* NP, 55%).
Perinatal protein restriction impaired mAChR's function, which might be an expression of decreased amount of mAChRM3 and/or alteration of signal transduction in β-cell from adult rats.
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PIII-354

Effect of Placental Vascular Restriction on Hepatic Cytochrome P4501A1 in near Term Fetus. Barent DuBois[1,2], Victoria H.J. Roberts[3], Peta L. Grigsby[3], Ganesh Cherala[1,2]. *[1]College of Pharmacy, Oregon State University, OR, USA; [2]College of Pharmacy, Oregon Health & Science University, OR, USA; [3]Division of Reproductive Sciences, Oregon National Primate Research Center, Oregon Health & Science University, OR, USA.*
While the effect of an adverse *in utero* environment on disease in later life is relatively well understood, the effectiveness of drugs employed in disease treatment is minimally studied. The CytochromeP450 (CYP) super-family of enzymes is primarily involved in detoxifying xenobiotics and/or generating toxic metabolites. The fetus is protected from maternal xenobiotic exposure by CYP enzymes expressed in either the placenta or the fetal liver. CYP1A1 is an important CYP isozyme involved in the metabolism of Theophylline; a drug routinely used in the treatment of apnea in prematurely born infants. Placental insufficiency is a leading cause of premature delivery. Therefore, our aim was to examine fetal liver and placental expression and activity of CYP1A1 in a unique nonhuman primate model of placental vascular restriction.
Inter-placental bridging vessel ligation (IPVL) surgery was performed in time-mated pregnant rhesus monkeys at either 80 days gestation (dGA, Early, n=4, term is 168 days) or 110dGA (Late, n=3). Control animals (n=3) were obtained from another cohort. Placental and fetal liver tissues were harvested following C-section delivery and fetal necropsy performed at 140dGA. Placental and fetal liver microsomes were prepared using differential centrifugation, and protein content determined using the Bradford assay. The microsomal CYP1A1 activity was measured by incubating microsomes with ethoxyresorufin and NADPH regenerating system and monitoring the formation of resorufin at excitation/emission wavelengths of 530/590 nm. The hepatic CYP1A1 activity in the early IPVL fetuses (15.2±7.6 fmoles/min/mg of protein) was similar to that of control animals (12.1±6.9 fmoles/min/mg of protein). In contrast, the late IPVL fetuses had higher activity (26.5±9.1 fmoles/min/mg of protein). Placental microsomal CYP1A1 activity was undetectable in all three groups.
Restriction of placental vascular supply during later fetal life alters the activity of CYP1A1 in fetal liver. These findings will likely translate into altered exposure of drugs and/or their metabolites in the developing fetus and may impact treatment effectiveness for diseases in later life.

PIII-355

Selective Uterine Artery Branch Ligation Is Associated with Altered Fetal Liver Gowth Factor Expression in a New Mouse Model of Placental Insufficiency. Mounira Habli, Helen Jones, Khaled Omar, Sundeep Keswani, Foong Yen Lim, Timothy Cromblehome. *Pediatric Surgery, Fetal and Molecular Therapy, Cincinnati Children Hospital, OH, USA.*
Previous work in our laboratory demonstrated that selective uterine artery branch ligation in a mouse model of intrauterine growth restriction (IUGR) significantly reduces fetal weight with no change in placental weight. In humans IUGR is associated with increased risks of hypertension, obesity and type 2 diabetes in later life but the mechanisms underlying this finding remain unknown. Following uterine artery ligation in the Wigglesworth model in the rat changes in fetal liver growth factor levels have been reported.
It has been suggested that these alterations may contribute to an increased risk of diabetes in later life. Therefore, we investigated the expression of IGF-1, IGF-2, IGFBP-1, PlGF, PDGF-B and PDGF-Rb in the fetal liver following selective uterine artery branch ligation in mice.
At E18, animals were divided into two groups; sham-operated controls or selective uterine artery branch ligation (ligated). At E20, pups and placentas were harvested by C-section. Fetal livers were removed by meticulous dissection and RNA extracted for growth factor expression analysis by qPCR, data presented as mean ± SEM, and analysed using Student's t-test.
IGF-1, IGF-2, PlGF and PDGF-Rb levels were the same as the sham-operated group following ligation; however there was a significant increase in the expression of IGFBP-1 in the livers of the ligated group compared to sham (1.32±0.19vs 0.53±0.06,p<0.05, n>4 per group)). In contrast, there was a significant reduction in PDGF-B expression in the livers of the ligated group compared to sham-operated controls (0.37±0.09 vs 1.16±0.11, p<0.01, n>3 per group).
Increased IGFBP-1 levels may reduce the bioavailability of the IGFs leading to alterations in liver development or function; furthermore reduced levels of PDGF-B in the liver have been associated with improper hepatic vascular delivery of insulin. The changes seen in fetal liver growth factor expression may represent an underlying mechanism that links IUGR with adult diseases such as diabetes.

PIII-356

Withdrawn by Author

PIII-357

Perinatal Salt Restriction: A New Model of Programming Low Birth Weight and beta-Cell Dysfunction in Adult Wistar Rats. Armando F. Vidonho Jr.[1], Karen L. Lopes[1], Laila R.B. dos Santos[2], Marlene S. Rocha[2], Luzia N.S. Furukawa[1], Michella S. Coelho[1], Angelo R. Carpinelli[2], Miriam S. Dolnikoff[1], Joel C. Heimann[1]. *[1]Department of Internal Medicine, University of Sao Paulo School of Medicine, Sao Paulo, Brazil; [2]Department of Physiology and Biophysics, Institute of Biomedical Sciences of the University of Sao Paulo, Sao Paulo, Brazil.*
Several studies support the hypothesis that chronic diseases in adult life might be triggered by events occurring during fetus life. Previous data have shown that maternal salt restriction during the perinatal period is associated with low birth weight and insulin resistance in adult offspring (Vidonho Junior AF *et al.* 2004). In the present study we evaluated the beta-cell function in the same experimental model.
Female Wistar rats were fed a normal (NS: 1.3%) or low (LS: 0.15% NaCl) salt diet from 8 wk of age until their offspring weaning. At 12 wk of age, they were matched with males fed NS. Birth weight, neonatal blood glucose (G) and weekly body weight were determined. At 12 wk of age the offspring were decapitated for blood and pancreas collection. G and insulin (INS) concentrations were determined. INS secretion was studied in offspring pancreatic islets extracted as described by Lacy *et al.*, 1967 and incubated in 5.6, 11.1 and 16.7 mM of glucose. In some experiments KCl or α-ketoisocarpoate (KIC) were added to 5.6mM of glucose to stimulate INS secretion**.** DNA fragmentation assay to evaluate apoptosis and glucose decarboxylation were also performed. Comparisons between groups were performed by one or two-way ANOVA followed by post-hoc test or by Student's t-test.
(mean±SEM, p<0.05): Birth weight and neonatal G were lower in offspring from LS dams than in controls. At 12 wk of age no differences were found between LS and NS offspring in fasting G and INS. No difference was observed in INS secretion from pancreatic islets in response to 5.6 mM of glucose. However, a lower INS secretion was observed in adult offspring from LS dams in response to 11.1 and 16.7 mM of glucose, compared to NS. After KCl and KIC stimulation, INS secretion increased, respectively, 200 and 445% in adult offspring from NS dams. In LS offspring INS secretion increased 330% in response to KIC but not to KCL. No differences between groups were observed in DNA fragmentation and glucose decarboxylation.
These results demonstrate that perinatal salt restriction impaired birth weight and beta-cell function in adult offspring. Supported by FAPESP.

PIII-358

Large Molecule Protein in Milk Is Significant for Cholecystokinin Secretion and the Development of the Exo/Endocrine Pancreas in Suckling Mammals. Daisuke Murakami[1], Toshi Kinouchi[1], Seiko Hoshi[1], Tetsuo Kaneko[1], Yuji Sunden[2], Tomohisa Tanaka[2], Takashi Umemura[2], Takashi Takeuchi[3], Etsumori Harada[3]. *[1]Food Science Research Labs, Meiji Co., Ltd., Japan; [2]Graduate School of Veterinary Medecine, Hokkaido University, Japan; [3]School of Veterinary Medicine, Faculty of Agriculture, Tottori University, Japan.*

Most amino acids in breast milk exist as the constituent of large molecule protein which infants need to digest. Our previous studies using rats suggest the intake of large protein in infancy is critical for proper pancreatic functions after weaning. We thus evaluated the physiological significance of stimulation by dietary protein-to-digest for the cholecystokinin endocrine cells and the exo/endocrine pancreas at the developmental stages, using a piglet model.

We first examined if dietary protein can stimulate cholecystokinin secretion in dam-fed piglets at 19 days of age, using a standard formula with milk protein and a protein hydrolysate formula (molecular weight: <3,500) designed for piglets. Then, piglets were artificially reared on either of the formulas from seven to 21 days of age. Blood samples were taken at 19 days of age and blood and tissue samples were taken at 21 days of age.

In dam-fed piglets at 19 days of age, oral administration of standard formula significantly raised plasma cholecystokinin concentration for over 40 minutes, whereas the hydrolysate formula did not increase plasma cholecystokinin. The AUC after the hydrolysate formula administration was significantly low, compared with the standard formula administration. Pancreas weights and digestive enzymes in the pancreas in piglets raised on the hydrolysate formula were significantly low, compared with standard formula-fed piglets at 21 days of age. The basal plasma cholecystokinin level was significantly low in hydrolysate formula-fed piglets. The plasma insulin level in hydrolysate formula-fed piglets at 19 days of age was significantly low, whereas pancreatic insulin concentration in hydrolysate formula-fed piglets at 21 days of age was significantly high, compared with standard formula-fed piglets. The results are consistent with previous rat studies.

These findings suggest that the stimulation by dietary protein during the suckling period may be critical for the proper development of intestinal and pancreatic functions in various mammals.

PIII-359

Does Oxidized LDL Impair Fetal Beta Cell Function? Zhong-Cheng Luo[1], Anne-Monique Nuyt[2], William Fraser[1], Pierre Julien[3], Edgard Delvin[2], Emile Levy[2]. *[1]Obstetrics and Gynecology, University of Montreal, Canada; [2]University of Montreal, Canada; [3]Laval University, Canada.*

Oxidized low density lipoprotein (OxLDL), a product of lipid peroxidation, has been implicated in the development of type 2 diabetes. It is unknown whether OxLDL may affect fetal beta cell function.

In a prospective study of 228 singleton non-diabetic pregnancies, we assessed fetal insulin sensitivity and beta cell function indicators (cord plasma glucose/insulin ratio, proinsulin/insulin ratio, HOMA insulin resistance and beta cell function index) in association with oxidative stress biomarkers [OxLDL, malondialdehyde (MDA), glutathione disulfide (GSSG) to glutathione (GSH) ratio] in maternal and fetal circulations.

Maternal and fetal plasma concentrations were strongly positively correlated in both OxLDL and MDA (r: 0.37 to 0.64, $p<0.001$). Cord plasma OxLDL concentrations were positively correlated with cord plasma glucose/insulin ($r=0.27$, $p<0.001$) and proinsulin/insulin ($r=0.20$, $p<0.01$) ratios and negatively correlated with HOMA beta cell function indices ($r=-0.27$, $p<0.001$). Comparing the highest vs. other quartiles in cord plasma OxLDL concentrations controlling for maternal, metabolic and delivery characteristics, cord plasma glucose concentrations were 6.6 mg/dl higher (adjusted $p=0.01$), insulin concentrations were 7.2 pmol/l lower (adjusted $p=0.01$), while HOMA beta cell function indices 21% lower (adjusted $p=0.002$). Other oxidative stress biomarkers were not associated with fetal beta cell function indices.

Maternal OxLDL levels affect fetal OxLDL levels, and OxLDL may impair fetal beta cell function and "program" glucose metabolism.

PIII-360

Hepatic and Pancreatic Innervation Are Altered by a Maternal High Fat Diet in the Non-Human Primate. Wilmon F. Grant[1,2], Lindsey E. Nicol[1,2], Sarah M. Comstock[3], Kevin L. Grove[3], Susan Smith[3], Daniel L. Marks[2]. *[1]Grant and Nicol Share First Authorship, USA; [2]Pediatrics, Oregon Health & Science University, USA; [3]Oregon National Primate Research Center, Oregon Health & Science University, USA.*

Previous studies in our nonhuman primate (NHP) model demonstrate exposure to a maternal high fat diet (HFD) *in-utero* induces nonalchoholic fatty liver disease, increased hepatic apoptosis and decreased plasma N3 fatty acids in the fetus. After partuition, evidence of insulin resistance and changes in body composition develop early with ongoing HFD exposure. The autonomic nervous system plays an important role in metabolic homeostasis in the liver and the pancreas. Additionally, the vagus nerve dampens peripheral inflammation via the cholinergic anti-inflammatory pathway and the nicotinic acetylcholine receptor α7 subunit (CHRNA7). Our aim is to describe changes in the innervation of the pancreas and liver in NHP offspring chronically exposed to HFD from conception to one year of age. In addition, we will also describe CHRNA7 expression in the liver and pancreas and examine its relationship to the development of insulin resistance in HFD juvenile offspring.

Liver and pancreas were harvested from juvenile rhesus macaques at 13 months of age. Our nomenclature in our cohorts first lists the diet exposure prior to weaning, the second to the post weaning diet (6 months). This produces four juvenile cohorts, CTR/CTR, HFD/HFD, CTR/HFD and HFD/CTR for analysis. Sympathetic nerve fiber density was quantified by immunohistochemisty and acquisition of 3-D digital images by confocal microscopy. Relative expression of CHRNA7 between cohorts was determined by qPCR.

There was a decrease in sympathetic innervation in the HFD/HFD males in portal and parenchymal regions. Quantification of nerve fiber density in the juvenile pancreas is ongoing. Decreased expression of CHRNA7 was also observed in the HFD/HFD juvenile liver. In the pancreas CHRNA7 expression was decreased in all three cohorts exposed to HFD.

The decrease in hepatic sympathetic innervation and CHRNA7 expression in both the liver and pancreas could render the animal susceptible to local inflammation promoting the development of insulin resistance observed in the HFD/HFD offspring. Data from the diet reversal groups implicates prenatal and post-weaning HFD exposures as independent but not additive risks of decreased CHRNA7 expression in the juvenile pancreas.

PIII-361

Pancreatic and Islet Vascularity Decline to a Greater Extent Than β-Cells in Intrauterine Growth Restricted Fetuses. Paul J. Rozance[1], Marina G. Martinez[2], Anna Fahy[2], Miranda Anderson[2], Sean W. Limesand[2]. *[1]Pediatrics, University of Colorado, CO, USA; [2]Animal Sciences, University of Arizona, AZ, USA.*

Pancreatic islet vasculature promotes β-cell development and function. Fetuses with severe intrauterine growth restriction (IUGR) have decreased β-cell function and mass and abnormal vascularity might contribute to these outcomes. Our objective was to measure pancreas and islet vascularity in IUGR sheep fetuses.

Placental insufficiency induced IUGR was created by exposing pregnant ewes with twins to elevated ambient temperatures (40°C for 12h; 35°C for 12h) during midgestation (40-110; term=148 days gestational age). In vivo fetal insulin secretion was measured with a hyperglycemic clamp. Pancreata were collected at 132 dGA (n=5, Control; n=5, IUGR). Pancreatic vasculature was identified with FITC conjugated Griffonia simplicifolia Agglutinin, which co-stained with von Willebrand factor. The fraction of vasculature$^+$ area was determined for the whole pancreas and islets. Endocrine cells were identified using antiserum raised against insulin, glucagon, somatostatin, and pancreatic polypeptide and detected with fluorescent secondary antibodies conjugated to AMCA (anti-insulin) or TexasRed (for other endocrine cells). β-cell mass was calculated by multiplying the insulin$^+$ area fraction by the pancreas weight.

Insulin secretion was lower in IUGR fetuses ($P<0.05$). Fetal weight was 48% lower ($P<0.05$, 1.5±0.1 kg vs. 2.9±0.2 kg) and pancreas weight was 55% lower in IUGR ($P<0.05$, 1.5±0.1 g vs. 2.8±0.2 g). Pancreatic vessel density was 37% lower ($P<0.05$, 3.38±0.63% vs. 5.36±0.32%) and islet vessel

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density was 42% lower in IUGR (P<0.05, 11.05±2.63% vs.18.96±1.98%). The insulin$^+$ area was not different (1.9±0.2% IUGR vs. 2.3±0.5% control). The β-cell mass, however, was 56% lower in IUGR fetuses (P<0.05, 29.2±2.5 mg vs. 65.9±18.9 mg). Glucagon, somatostatin, and pancreatic polypeptide combined positive area was not different (2.0±.3% IUGR vs. 2.2±0.6% control).

Pancreas and islet vascularity were reduced to a greater extent than insulin$^+$ or other endocrine cell areas. These data can partially explain the impaired insulin secretion given the importance for the endothelium in promoting β-cell proliferation and function.

PIII-362

Maternal Overnutrition and Weight Loss in the Periconceptional Period Lead to Differential Changes in Expression and Epigenetic Modification of IGF2 & IGF2R in Liver and Skeletal Muscle of the Offspring. L. Nicholas[1], L. Rattanatray[1], J. Morrison[1], S. Zhang[1], S. McLaughlin[1], D. Kleeman[2], S. Walker[2], C. Suter[3], C. McMillen[1]. *[1]Sansom Institute for Health Research, Australia; [2]Turretfield Research Centre, SARDI, Australia; [3]Victor Chang Cardiac Research Institute, Australia.*

The imprinted gene, IGF2, plays a key role in growth and metabolism. This study investigated the impact of maternal periconceptional overnutrition (PCON) with or without a period of dietary restriction on DNA methylation in the proximal CTCF-binding site in the differentially methylated region (DMR) of IGF2/H19 gene and IGF2 and IGF2R expression in liver and muscle of the offspring. We hypothesized that PCON would lead to increased IGF2/H19 methylation and IGF2 expression and decreased IGF2R expression in liver and muscle of lambs and that dietary restriction in the overnourished ewe would abolish these effects.

Donor ewes were allocated to one of four nutritional treatment groups pre-conception; CC group-100% metabolizable energy requirements (MER) from four mos. CR group-100% MER for three mos preceding 70% MER for one mo. HH group-ad libitum (~180% MER) from four mos. HR group-ad libitum for 3mos preceding 70% MER for 1mo. At 6-7d post-conception, single embryos were transferred into normal weight recipient ewes. Post mortem was conducted on offspring at four mos. Expression of IGF2 and -2R mRNA and protein expression was determined by qRT-PCR and SDS-Page respectively. DNA methylation within the DMRs of IGF2/H19 and IGF2R was determined by combined bisulphite restriction assay.

Hepatic IGF2 mRNA expression was higher (P<0.05) in male lambs. IGF2 protein abundance was lower (P<0.05) in the liver of both male and female lambs in HH compared to the CC group. Muscle IGF2 mRNA and protein expression in male and female lambs, however, was higher (P<0.05) in the HR compared to the CC group. IGF2R mRNA expression and protein abundance was also higher (P<0.05) in muscle of lambs in HR compared to the CC group. In contrast, there was no effect of dietary restriction in overnourished ewes on hepatic IGF2R expression in lambs. Methylation of both hepatic and muscle IGF2/H19 DMR was lower (P<0.05) in the CR group.

These results suggest that both maternal high and low starting body weight and maternal metabolic response to dietary restriction in the periconceptional period have different but long lasting consequences for epigenetic programming and signalling of IGF2 and IGF2R in the offspring.

PIII-363

Fasting Plasma Glucose in Early Pregnancy Is Associated with Slow Intrauterine Growth in Late Pregnancy. Marie Cecilie P. Roland[1], Camilla M. Friis[1], Nanna Voldner[1], Kristin Godang[2], Jens Bollerslev[2,3], Guttorm Haugen[1,3], Tore Henriksen[1,3]. *[1]Department of Obstetrics and Gynecology, Oslo University Hospital, Norway; [2]Department of Endocrinology, Oslo University Hospital, Norway; [3]University of Oslo, Norway.*

The aim of this study was to explore the associations between maternal characteristics and deviating fetal growth patterns in late pregnancy.

A prospective study including 1031 healthy Caucasian women. Maternal characteristics were parity, BMI measured at weeks 14-16, weight gain during pregnancy and fasting plasma glucose levels at gestational week 14-16.

Fetal growth was analyzed by a conditional growth model. Growth was calculated for the period between weeks 30-32 and weeks 36-38 using estimated fetal weight at weeks 30-32 as baseline. Slow fetal growth was defined as fetal growth < 10 conditional percentile and accelerated fetal growth as > 90 conditional percentile for that period.

Associations between maternal characteristics and deviating fetal growth were explored by multinomial logistic regression, comparing slow and accelerated fetal growth with normal fetal growth.

Accelerated fetal growth during this period was associated with parity (P1 vs P0; OR 2,9; 1.6,5.1, p<0,001) and BMI of the mother (OR1,1; 1.04,1.17, p=0,001).

Slow fetal growth was associated with BMI (OR 0,93; 0.88,0.99, p=0,023), weight gain during pregnancy (OR 0,91; 0.85,0.96, p=0,001) and fasting plasma glucose levels in weeks 14-16 (OR 0,64; 0.41, 0.98, p=0,042).

We have identified determinants of deviating fetal growth. Our results indicate that these determinants differ from those of small for gestational age (SGA) or large for gestational age (LGA) newborns.

BMI is a determinant of both accelerated and slow fetal growth. Parity is associated with accelerated fetal growth. Whereas both weight gain and plasma glucose are known to be determinants of LGA newborns, we did not identify these as determinants of accelerated fetal growth. However, both weight gain and plasma glucose levels are significantly negatively associated with slow intrauterine fetal growth in late pregnancy.

PIII-364

Assisted Reproduction Associated with Increased Glucose Levels at Age 5: The Amsterdam Born Children and Their Development Study. Tessa J. Roseboom[1], Rebecca C. Painter[1], Marcel Van der Wal[2], Joris A. Van der Post[1], Tanja Vrijkotte[3]. *[1]Obstetrics and Gynaecology, Academic Medical Center Amsterdam, Netherlands; [2]Public Health Service Amsterdam, Netherlands; [3]Public Health, Academic Medical Center Amsterdam, Netherlands.*

The environment around the time of conception affects the offspring's growth and cardiovascular physiology. There are indications that assisted reproduction techniques – that are increasingly being used in the Western world – may have lasting consequences for health. We here investigate effects of assisted reproduction techniques (ART) on metabolic profiles of five year old children.

The Amsterdam Born Children and their Development study (www.abcd-study.nl) is a large multi-ethnic cohort study that was set up in 2003 to include all pregnant women in Amsterdam in that year. Detailed information on life style, psychological conditions, nutrition and socio-demographic background was recorded and these women as well as their children are followed. 3263 children were examined at the age of five years. Using linear regression analyses we compared the metabolic profiles of children who had been conceived through ART (IUI, IVF or ICSI as reported by their mothers during pregnancy) with that of those who had been conceived naturally.

131 children out of the 3263 (4%) had been conceived through ART. Birth weights of ART conceived children were not different from that of those who were conceived naturally. Their weight and height did not differ at age 5, nor did their lipid profiles (p > 0.2). Glucose levels were higher among ART conceived children compared to those who had been conceived naturally (0.19 mmol/l, 95% CI 0.06 to 0.28). Adjustments for differences in maternal characteristics (ethnicity, socio-economic status, maternal age, diabetes gravidarum, hypertension, maternal weight and height) did not explain these differences (adjusted difference 0.17 mmol/l 95% CI 0.06 to 0.28).

Children conceived through ART had higher glucose levels at age five compared to naturally conceived children. This may suggest that assisted reproduction techniques have lasting consequences for infant's health without affecting size at birth. Optimizing the early environment of individuals conceived through these techniques (through optimizing culture media/hormone stimulation etc) may provide a new challenge in the prevention of disease in future generations.

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PIII-365

Pre-Conceptional Maternal Nutrition Determines Levels of Key Pancreatic Genes in the Leptin Signalling Pathway. Graham J. Howie, Deborah M. Sloboda, Mark H. Vickers. *Liggins Institute and NRCGD, Univ. of Auckland, New Zealand.*

A maternal high fat diet results in obesity and metabolic disorders in offspring, independent of post-weaning diet. Although obesity occurs independently of pre-conceptional maternal diet we now show that key genes regulating the pancreatic adipoinsular axis have altered mRNA levels which is dependent upon pre-conceptional maternal nutrition.

Female Wistar rats were randomized to one of three groups: 1) Control (CONT): dams fed a standard diet pre-conceptionally and during pregnancy and lactation; 2) Maternal High Fat (MHF): a HF diet fed pre-conceptionally and during pregnancy and lactation; 3) Pregnancy and Lactation High Fat (PLHF): a HF diet fed during pregnancy and lactation only. Body composition of dams was determined by DEXA prior to mating. Male offspring were fed a control diet from weaning. Adult offspring body composition was assessed by DEXA and blood and pancreatic tissue were collected. Relative mRNA levels of key genes in the leptin and insulin signalling pathways were determined by qPCR.

MHF dams had increased total fat mass at mating compared to CONT and PLHF dams. MHF and PLHF offspring were heavier than CONT offspring from weaning until P180, had increased % body fat and were hyperinsulinemic and hyperleptinemic compared to CONT. The relationship between key signalling factors was altered between PLHF and CONT offspring: in PLHF offspring PI3K mRNA levels positively correlated with IRS1 and IRS2 mRNA but not in CONT. Leptin receptor (ObRb) mRNA levels were similar to CONT despite hyperleptinemia, suggesting a down-regulation of receptor numbers in PLHF offspring. In contrast, MHF offspring had increased levels of ObRb and its downstream signalling factors, SOCS3 and IRS1. In MHF offspring, ObRb and SOCS3 were positively correlated and SOCS3 was positively correlated with IRS1, IRS2, K+ channel, and Pdx1 mRNA in both MHF and CONT, but not in PLHF offspring. However, Pdx-1 was positively correlated with STAT3, Ins1, Ins2 and IR mRNA only in PLHF offspring, and not in MHF or CONT offspring.

MHF and PLHF male offspring exhibited distinct expression patterns for key genes in pancreatic leptin signalling pathways that were dependent upon maternal pre-conceptional nutrition. These data suggest that factors associated with maternal adiposity prior to pregnancy can influence pancreatic gene expression in offspring, and may be linked to metabolic compromise.

PIII-366

Maternal Pre-Pregnancy Body Mass Index, Birth Weight and Blood Pressure in the Child at Age 5. Tanja Vrijkotte[1], Marcel van der Wal[2], Joris van der Post[3]. *[1]Public Health, Academic Medical Center, Amsterdam, Netherlands; [2]Education, Documentation and Health promotion, Public Health Service Amsterdan, Amsterdam, Netherlands; [3]Obstetrics and Gynecology, Academic Medical Center, Amsterdam, Netherlands.*

The intra uterine nutritional status influences birthweight and cardio vascular health later in life. One of the underlying mechanisms could by alterations in cardiac autonomic balance, programmed already *in-utero*. Our aim was to study the association of pre-pregnancy body mass index (BMI) and birth weight with blood pressure and heart rate (HR) in the child at age five and with respiratory sinus arrhythmia (RSA; a measure of parasympathetic drive) and pre ejection period (PEP; a measure of sympathetic drive).

Pregnant women participating in the Amsterdam Born Children and their Development (ABCD) study completed a questionnaire at gestational week 14 of their index pregnancy including information on demographics and pre-pregnancy weight and length.. At age 5, systolic and diastolic blood pressure (SBP, DBP) as well as PEP, RSA and HR were measured by electrocardiography and impedance cardiography in resting supine and sitting position. Maternal BMI was divided into four groups: BMI < 18.5 kg/m2 (underweight), BMI 18.5-25 kg/m2 (normal weight; reference group), 25-30 kg/m2 (overweight) and > 30 kg/m2 (obese). Birth weight was categorized into small for gestation age (SGA), appropriate for gestational age (AGA: reference group) and large for gestational age (LGA) according to national references. Only mothers with singletons, not using anti-hypertensive medication and children without reported heart problems were included (N=3,148). Results were adjusted for gender, child's age and BMI.

Maternal pre-pregnancy obesity was associated with increased DBP in the child (1.50;SE: 0.60 mmHg) and increased HR (1.79;SE: 0.81 beats/minute). Birth weight did not mediate these associations; however it had an independent effect. SGA children had higher DBP (0.97;SE:0.46 mmHg), higher SBP (1.51;SE 0.49 mmHg) and higher HR (1.39;SE:0.62 beats/minute). Pre-pregnancy BMI and birth weight were not associated with RSA and PEP.

Pre-pregnancy obesity and intra uterine growth retardation are associated with higher blood pressure and heart rate in the child at age 5, independently of child's current BMI. This seems not to be the result of early alterations in cardiac autonomic balance.

PIII-367

Maternal Periconceptional Undernutrition and Fetal Number Have Differential Effects on TSH, PRL and GH mRNA Expression in the Anterior Pituitary of the Late Gestation Sheep Fetus. Song Zhang[1], Olivia Wyss[1,2], Isabella Caroline McMillen[1], Janna L. Morrison[1]. *[1]Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia, SA, Australia; [2]Discipline of Physiology, School of Health Sciences, University of Adelaide, SA, Australia.*

The aim of the present study was to investigate the effects of exposure of the oocyte and/or the embryo to maternal undernutrition on the synthetic capacity of thyrotrophs, lactotrophs, somatotrophs and gonadotrophs in the anterior pituitary of the sheep fetus.

We have compared the effects of periconceptional undernutrition (PCUN: maternal undernutrition imposed from at least 45 days before until six days after conception), and early preimplantation undernutrition (PIUN: maternal undernutrition imposed for only six days after conception) on the expression of thyroid-stimulating hormone (TSH), prolactin (PRL), growth hormone (GH), follicle stimulating hormone (FSH) and luteinizing hormone (LH) mRNA as assessed by quantitative real time RT-PCR in the fetal sheep anterior pituitary at 136-138 days gestation. Both singleton (Control n=6, PCUN n=8, PIUN n=3) and twin fetuses (Control n=9, PCUN n=6, PIUN n=9) were included in the study.

TSH mRNA expression was significantly higher ($P < 0.05$) in the anterior pituitary of fetuses in the PCUN group compared with both the control and PIUN groups. Pituitary PRL mRNA expression was higher ($P < 0.05$) in the PIUN group when compared with the control and PCUN groups. There was no effect of either PCUN or PIUN on pituitary GH, FSH and LH mRNA expression in the late gestation fetus. Pituitary PRL mRNA expression was lower ($P < 0.01$) while GH mRNA expression was higher ($P < 0.05$) in twins compared to singletons. Pituitary FSH mRNA expression was significantly higher in females compared to males ($P < 0.05$) whereas LH mRNA expression was higher ($P < 0.01$) in males compared to females.

Our results suggest that there are critical windows during which poor maternal nutrition has a significant impact on the synthetic capacity of the thyrotrophs (during the period of oocyte and early embryo development) and the lactotrophs (during early embryo development). In contrast the reciprocal pattern of pituitary PRL and GH mRNA expression in twins and singletons indicate that either being a twin embryo or the relatively lower placental substrate supply in twins results in changes in either differentiation or activation of the fetal lactotrophs and somatotrophs.

PIII-368

Differential Expression of A and B Isoforms of Insulin Receptor Are Involved in the Insulin-Mediated Recovery of Gestational Diabetes to Normal Phenotype in Human Placental Microvascular Endothelium. Carlos Salomon, Francisco Westermeier, Enrique Guzman, Andrea Leiva, Paola Casanello, Sobrevia Luis. *Cellular and Molecular Physiology Laboratory (CMPL) & Perinatology Research Laboratory (PRL), Division of Obstetrics and Gynecology, Faculty of Medicine, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile.*

Functional equilibrative nucleoside transporters 1 (hENT1) and 2 (hENT2), and insulin receptor isoforms A (IR-A) and B (IR-B) are expressed in human placental microvascular endothelium (hPMEC), but insulin effect on

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adenosine transport in this cell type, and potential GD effects, are unknown. We studied the involvement of IR-A and IR-B on hENTs-mediated adenosine transport in hPMEC from GD.

hPMEC were isolated from full-term normal or GD pregnancies and cultured under standard conditions. hENTs-mediated adenosine transport (0-500 μM adenosine, 2 μCi/ml [3H]adenosine), protein abundance and mRNA expression, stability and promoter activity of SLC29A2 were determined by Western blot, Q-PCR and luciferase assay, respectively. IR-A and IR-B mRNA expression were also quantitated by Q-PCR. An adenoviral-based siRNA delivering system was used to knockdown IR-A (siIR-A) and IR-B (siIR-B) expression.

GD is associated with reduced hENT1 (70%) and hENT2 (80%) mediated adenosine transport. Insulin increased hENT2 mediated adenosine transport by 5-fold in GD and 1.2-fold in normal hPMEC. This effect of Insulin was paralleled by the recovery of hENT2 expression (protein and mRNA), without changes in protein or mRNA stability, and reverses the GD-reduced SLC29A2 (for hENT2) promoter activity. Basal IR-A mRNA expression was lower (65%), and IR-B was higher (2.0-fold) in GD compared with normal hPMEC. In normal hPMEC, insulin increased IR-A (1.9-fold) without changes in IR-B mRNA expression. However, in GD insulin increased IR-A (1.4-fold) and recovered IR-B to values observed in normal hPMEC. The effect of insulin in GD hPMEC was abolished in siIR-B, but not in siIR-A hPMEC.

These changes found in placental microvascular endothelium from GD pregnancies reveal the importance of the subtype of insulin receptors involved in mitogenic (IR-A) and metabolic (IR-B) pathways, and the effects of this maternal disease in the programming of vascular function in the placenta.

PIII-369

Ontogeny of Glucocorticoid Receptor Alpha Positive Stained Binucleate Cells in Sheep Placenta. H. Shang[1], K. Lange[1], W. Meng[1,6], D. M. Sloboda[2], S. Li[4], A. Plagemann[1,3], J. W. Dudenhausen[1], J. P. Newnham[4], J. R.G. Challis[5], T. Braun[1,3]. *[1]Department of Obstetrics Charité Berlin, Germany; [2]The Liggins Institute University of Auckland and The National Research Centre for Growth and Development, New Zealand; [3]Division of Perinatal Programming Charité Berlin, Germany; [4]School of Women's and Infants' Health UWA, Australia; [5]Michael Smith Foundation for Health Research, Canada; [6]The Affiliated Hospital of Inner Mongolia Medical College, China.*

We have previously shown in sheep that the activation of the fetal HPA axis and a concurrent increase in plasma cortisol concentrations sig. decreased placental binucleate cells (BNC) which produce the key hormone for fetal growth. Since the effects of cortisol on intrauterine tissues are mediated, in part, by glucocorticoid receptors (GR), we hypothesized that cortisol-mediated changes in placental BNC number are mediated by GR alpha (GR-a), the functional isoform of GR.

Pregnant ewes carrying singleton fetuses (total n=51) were included. Placental tissue was collected at 50, 100, 125, 140 days of gestations (dG). The temporal and placentome subtype- and tissue-specific distribution of GR-a positive stained BNCs were analysed regarding the placentome subytpes (ps) and within three levels L1-3, which refer to previously reported zones in a placentome.

GR-a positive staining was localized to uninucleate and binucleate cells and was independent of gestational age, fetal sex and ps. The mean number of GR-a positive BNCs increased from 50 to 100 dG and then decreased to 125 dG. This decrease was observed mainly in pregnancies with male fetuses. Three different GR-a staining patterns were found: BNCs with two GR-a positively stained nuclei (++), BNCs lacking any GR-a staining in the nuclei (--) and BNCs with one positive and one negative GR-a nucleus (+-). (++) was the most frequent cell type, (--) the least frequent cell type. Across gestational age, the proportion of total of (++) decreased from 100 to 140 dG whereas the proportion of total of (--) increased towards term. There were no changes in the proportion of (+-). The highest number of (++) was found in L1 compared to L3.

We speculate that three different functional subtypes of BNCs exist with (++) as the active form, (+-) as the intermediate form and (--) as the inactive form and that the activity of BNCs is determined by the distribution of these three cell types. GR-a may play an important role in mediating the effect of cortisol on BNCs function and the conversion from the active to the inactive form.

PIII-370

Long Term Effects of Early Dexamethasone Treatment on Glucocorticoid Receptor Alpha and the Regulation of Binucleate Cell Function in Sheep Placenta. H. Shang[1], W. Meng[1,6], D. M. Sloboda[2], S. Li[4], A. Plagemann[1,3], J. W. Dudenhausen[1], J. P. Newnham[4], J. R.G. Challis[5], T. Braun[1,3]. *[1]Department of Obstetrics Charité Berlin, Germany; [2]The Liggins Institute University of Auckland and The National Research Centre for Growth and Development, New Zealand; [3]Division of Perinatal Programming Charité Berlin, Germany; [4]School of Women's and Infants' Health, UWA, Australia; [5]Michael Smith Foundation for Health Research Vancouver, Canada; [6]The Affiliated Hospital of Inner Mongolia Medical College, China.*

The effects of GC on fetal development and growth are mediated in part by GC receptors (GR). We have previously shown that GR-a, as the functional isoform, may play an important role in mediating the effect of cortisol on BNCs function. We have previously shown the presence of three different types of GR-a positive stained binucleate cells (GR-aBNCs) in sheep placenta and hypothesized that the effects of DEX on BNCs function and its conversions among these three cell types are mediated by changes in GR-a.

Pregnant ewes carrying singleton fetuses (n=101) were randomized to control (2 ml saline/ewe) or DEX treated groups (i.m. injections of 0.14 mg/kg ewe weight per 12 h) at 40–41 days of gestation (dG). Placental tissue was collected at 50, 100, 125, 140dG. The localization and distribution of GR-a BNCs was analyzed regarding placentome subytpes (ps) and within three levels L1-3, which refer to previously reported zones in a placentome.

DEX did not affect the localization of GR-a within the BNCs, three different cell types were present: BNCs with two GR-a positively stained nuclei (++), BNCs lacking any GR-a staining in the nuclei (--) and BNCs with one positive and one negative GR-a nucleus (+-). In males the mean number of GR-aBNCs was sig. decreased at 100 and 140dG compared to controls. At 100 dG, these changes were due to a decrease in all three cell types in A-ps; at 140dG due to a decrease in (++) and (+-) in C-ps in L1. In females, DEX sig. lowered the mean number of (++) at 125dG in B-ps compared to controls. The proportion of total of (++) after DEX sig. increased from 50 to 100 dG, whereas the proportion of total of (+-) and (--) sig. decreased.

Early DEX administration had long lasting effects on the mean number of GR-aBNCs which were sex- , cell type- and level dependent. Early DEX increased the conversion of BNCs from the active to the inactive form early in pregnancy, and may decrease the sensitivity of GR-a in BNCs near term to the natural occurring cortisol surge.

PIII-371

Maternal Fructose Intake in the Rat Induces Changes in Placental Weight and Nutrient Transporter Levels. Cassandra Yap[1], Mark H. Vickers[1], Zoe E. Clayton[1], Peter J. Mark[2], Deborah M. Sloboda[1]. *[1]Liggins Institute, University of Auckland and the NRCGD, New Zealand; [2]School of Anatomy & Human Biology, University of Western Australia, Australia.*

The increasing prevalence of obesity and metabolic syndrome is paralleled by the increasing intake of sugars. Despite widespread consumption of fructose-containing foods and beverages, little attention has been paid to possible adverse fetal effects of fructose intake during pregnancy. We therefore examined the effects of maternal fructose intake during pregnancy on placental and fetal development.

Pregnant Wistar rats received either a control (CON, n=10) or fructose diet (FR, n=9) throughout pregnancy. On embryonic day (E)21, maternal and fetal blood samples and placental tissue were collected. Blood glucose, ketone levels and plasma amino acids (AAs), fructose, insulin and leptin levels were determined. Placental levels of glucose (GLUT1, 3, 5) and AA transporters (SNAT 1, Taurine transporter; TAUT) and components of the mammalian target of rapamycin (mTOR, 4EBP1) were measured by Western Blot.

Maternal bodyweight, blood glucose, electrolyte and ketone levels did not differ between groups. FR dams had high plasma fructose and insulin levels and reduced levels of some amino acids. Placental weights were decreased in FR females but not males. Fetal weight was similar between groups and

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placental efficiency (fetal:placental weight ratios) was increased in FR females but not males. FR females had higher blood glucose, plasma fructose and leptin levels but lower ketones compared to CON; these effects were absent in FR males. Levels of valine and lysine were decreased in male and female FR fetuses, but levels of taurine were increased in FR males only. Females had decreased placental SNAT1 and GLUT1 levels whereas males had decreased GLUT3 levels. Placental mTOR levels were lower in FR males whereas 4EBP1 levels were lower in FR females. TAUT levels were similar between groups.

Maternal fructose intake during pregnancy resulted in sex-specific fetal compromise that appeared to be associated with changes in placental nutrient transport, but was not associated with fetal growth restriction. Changes in fetal plasma amino acid levels may be due to sex-specific changes in placental transfer; and it appears that female fetuses were more vulnerable to fructose-induced endocrine changes. In contrast, males appear protected and elevated levels of plasma taurine may indicate sex-specific compensatory mechanisms.

PIII-372

Ovine Uterine Space Restriction Altered Placental Transferrin Receptor (TfR) Expression: Interplay between Fetal Iron and the eNOS Pathway. Mary Y. Sun, Jason M. Habeck, Jill M. Koch, Katie Meyer, Ronald R. Magness, Pamela J. Kling. *Ob/Gyn, Pediatrics, Nutritional Sciences, University of Wisconsin, Madison, USA.*

In humans and rodents, transferrin receptor (TfR) is regulated by nitric oxide (NO)-associated iron regulatory proteins (IRP) 1 & 2. TfR-mediated placental iron transport is further controlled by fetal liver iron status. However, little is known if NO/endothelial NO synthase (eNOS) regulates placental TfR expression during normal or compromised (IUGR) pregnancies. We hypothesized that, in compromised pregnancies, placental TfR expression is altered and associated with eNOS expression.

Compromised IUGR sheep fetuses (USR) were produced with triplet or quadruplet gestations or twins in a space restricted uterus (single unilateral uterine horn ligation; Meyer et. al, BOR 2010). USR fetuses were compared to non-space restricted (NSR) controls at gestation day (GD) 120 and 130 (term=147). Fixed placentomes were stained in hematoxylin, DAB, and a CD-71 TfR antibody for immunohistochemistry (IHC). Immunoblotting was performed for TfR and eNOS expression. Liver tissue iron was quantified with a non-heme iron assay.

IHC and immunoblot showed TfR in both NSR and USR placentomes, but the most staining was at GD130 in the NSR group. eNOS immunoblot expression increased with gestation and was directly correlated with TfR expression (R2=0.468, p=0.0003). Additionally, the pattern of fetal liver iron content paralleled that of placentome TfR expression.

Our data support that, in addition to fetal liver iron, placental eNOS appears to be involved in the regulation of TfR expression. Investigation of placental iron transport should interrogate the interaction of eNOS and TfR in normal and compromised sheep pregnancies. NIH HL49210, HD38843, HL87144+Supplement

PIII-373

Maternal Influences on Placental Epigenetic Signatures in Pregnancies of Overweight and Obese Women. Julie A. Owens, Tulika Sundernathan, Anne MacPherson, Rosalie Grivell, Andrea Deussen, Jeffrey Robinson, Jodie Dodd, Jodie Dodd. *School of Paediatrics and Reproductive Health, University of Adelaide, SA, Australia.*

Childhood obesity has reached epidemic proportions world-wide [1]. A major risk factor for childhood obesity is maternal overweight and obesity, also increasingly common [2]. The association between maternal and childhood adiposity is likely to reflect a combination of shared genetic and other factors, including 'programming' of offspring by the intra-uterine environment. This may include maternal factors associated with obesity that act on the placenta to indirectly affect the latter. Altered epigenetic state is increasingly implicated in placental functional development, but has been little studied in maternal obesity to date. Maternal BMI is predictive of peroxisome proliferator activated receptor gamma coactivator 1α (*PPARGC1α*) methylation in the cord of LGA newborns [3], but the impact on DNA methylation of the gene for this major metabolic regulatory molecule in the placenta is unknown.

We therefore examined the influence of maternal BMI and related characteristics (maternal blood glucose, triglycerides, insulin) from mid gestation to term, in overweight and obese women, on DNA methylation in the promoter of *PPARGC1α* and a DMR of *IGF2*, in the placenta, collected at delivery in a subset of the LIMIT RCT (n=15). DNA was extracted, bisulphite treated and subjected to pyrosequencing.

Maternal BMI, plasma triglycerides or insulin were not associated with DNA methylation of either loci. Maternal blood glucose in mid- but not later in gestation, was positively associated with placental *PPARGC1α* methylation at four of five sites (r=0.73, p=0.007 to r=0.845, p=0.001). In contrast, no maternal measure was associated with methylation of sites in the *IGF2* DMR.

Maternal glycaemia in mid gestation influences methylation of *PPARGC1α* in the placenta in overweight or obese women. Increased maternal blood glucose increases methylation of this major transcriptional co-activator that regulates mitochondrial biogenesis and function. This may impair glucose and lipid homeostasis and alter placental function, exacerbating obesity related changes to the intrauterine environment of the developing fetus. This also suggests that maternal obesity can program placental epigenetic state via glycaemia early in pregnancy.

1. de Onis M *et al.* 2010 Am J Clin Nutr 92: 1257
2. Dodd JM *et al.* 2011 Maternal ANZJOG In press
3. Gemma C *et al.* 2009 Obesity 17: 1032

PIII-374

Mother's Lifetime Nutrition and the Size, Shape and Efficiency of the Placenta. Nicola R. Winder[1], Ghattu V. Krishnaveni[2], Sargoor R. Veena[2], Jacqueline C. Hill[1], Chitra L.S. Karat[2], Kent L. Thornburg[3], Caroline H.D. Fall[1], David J.P. Barker[1,3]. *[1]MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, United Kingdom; [2]Epidemiology Research Unit, CSI Holdsworth Memorial Hospital, Mysore, India; [3]Heart Research Center, Oregon Health and Science University, Portland, USA.*

Studies have shown that the shape and size of the placenta predict chronic disease in later life, but the influences that determine placental morphology are largely unknown. We hypothesized that mother's body size was one such influence.

Maternal height, head circumference, arm muscle area and fat mass were measured at 28-32 weeks gestation in 522 Indian women who gave birth to healthy, term babies in Mysore India in 1998-1999. Maternal birthweight was obtained from hospital records for a subset of 51 mothers. At delivery, the baby's birthweight, and placental length, breadth and weight were measured. Ratios of birthweight to placental dimensions were derived as indicators of placental efficiency.

Higher maternal fat mass predicted a larger placental surface (p=0.02), while larger maternal head circumference predicted a more oval placental surface (p=0.03). Greater placental efficiency, indicated by lower ratios of placental surface length and breadth to birthweight, were associated with higher maternal fat mass (p for the ratios =0.0003 and 0.0002) and larger maternal head circumference (p=0.0001 and <0.0001). Higher maternal birthweight was related to higher birthweight in the baby and lower ratios of placental surface length and breadth to birthweight (p=0.01 and 0.002). Maternal height did not influence placental size or shape.

High maternal fat mass may be associated with a large placental surface, and greater placental efficiency, because higher fat mass makes more lipids and glucose available to the placenta and fetus. Larger maternal head circumference may be associated with greater placental efficiency because it reflects the development of a denser spiral artery supply to the mother's decidua during her intra-uterine life.

PIII-375

Comparison of Two Models Exploring the Relationship between Early and Later Growth with Adult Risk Factors. A. Pande, C. Joglekar, C. Yajnik. *KEM Hospital Research Centre, MH, India.*

There has been a growing recognition of the life course evaluation of chronic non-communicable diseases. They have an early life origin and progressive amplification of the risk is related to childhood and adult growth. Analyzing such data is difficult due to these complex interrelationships. Two models have been suggested as the initial simplification: Lucas model (LM-1) and the unexplained residual model (URM-2). The LM uses the raw values

of later growth, ignoring the collinearity between early and later growth, whereas URM tries to overcome this by considering the difference of later and expected later growth from early growth. We compared the results of these models in the Pune Children Study which is a birth cohort with serial measurement of growth and cardiovascular risk factors.
We used serial weight measurements (birth, 8 yr and 21 yr) to predict fat mass at 21 yr.
The results of early model with birth weight (BW), are similar for both models (β=1.33). The results of late LM, with weight at 21 yr (CW) and URM, with weight gain (difference of CW and expected CW from the BW) also gives the similar results (LM: β=0.43, URM: β=0.45). In the combined LM, where both BW and CW are included, a substantial change in the BW regression coefficient was observed in comparison to early model (β=-1.90), but not in the combined URM (β=1.50) where BW and weight gain are taken. This discrepancy in the results of two models could be because the combined LM ignores the effect of BW on the CW, whereas combined URM accounts for this relationship. Inclusion of BW and CW in the same model is criticized because the effect of CW comes in the causal pathway between BW and outcome. Similar results were observed for the interaction models. When 8 yr weight was also included in combined LM, the regression coefficient for BW showed further decrease (β=-2.52), but not in the combined URM (β=1.43). This further deviation in the LM is possibly due to a stronger relationship between BW and 8 yr weight.
Conclusion: The difference of the result in the two models suggests different approach and therefore it should be interpreted accordingly. It should also promote a discussion about statistical interpretation of early life origin studies.
Ref
1. Lucas A *et al.*, BMJ 1999. Fetal origin of adult disease-the hypothesis revisited
2. M G Keijzer-Veen *et al.*, J Clin Epid 2005. A regression model with unexplained residual was preferred in the analysis of the fetal origins of adult diseases hypothesis.

PIII-376

Polymorphisms in Genes within the IGF-Axis Influence Antenatal and Postnatal Growth. P. G. Parmar[1], J. A. Marsh[1], R. H. Taal[2,3], J. P. Newnham[1], A. G. Uitterlinden[1], L. Briollais[5], S. J. Lye[5], L. J. Palmer[6], A. Hoffman[2], E. A.P. Steegers[4], C. M. van Duijn[2], V. W.V. Jaddoe[2,3], C. E. Pennell[1]. *[1]School of Women's and Infants' Heath, UWA, Australia; [2]The Gen R Study Group and Department of Epidemiology, Erasmus Medical Centre, Netherlands; [3]Department of Paediatrics, Erasmus Medical Centre, Netherlands; [4]Department of Obstetrics and Gynaecology, Erasmus Medical Centre, Netherlands; [5]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Canada; [6]Ontario Institute for Cancer Research, University of Toronto, Canada.*
The insulin-like growth factor (IGF) pathway is fundamental in cell proliferation, differentiation and transformation across all stages of growth and development. Two pregnancy cohorts were used to investigate the association between SNPs in genes within the IGF axis and antenatal and postnatal growth from birth until adolescence.
145 SNPs in IGF-1, IGF-2, their receptors and binding proteins were genotyped in the Raine Cohort. Longitudinal analyses in the Raine cohort were conducted using repeated measures of fetal head circumference (HC) and abdominal circumference (AC) from 18-38 weeks gestation and eight measures of postnatal BMI (1-17 years). Analyses modelling multiple SNPs simultaneously were performed for birth-weight. Analyses were replicated, where possible, in the Generation R Cohort (12-30 weeks gestation).
Of the 145 SNPs genotyped within the IGF-axis genes, 60 (41%) were associated with measures of antenatal growth. The majority of these SNPs were in receptors; IGF-1R (39) and IGF-2R (10). From the 17 most highly significant SNPs in Raine ($p \leq 0.005$), six replicated in GenR. Associations were identified between 39 of the 145 IGF-axis SNPs and postnatal BMI. Of these, 17 SNPs were associated with both antenatal and postnatal growth; 12 with discordant effects and five with concordant effects. When modelling 3 SNPs simultaneously, remarkably, the presence of four or more adverse alleles was associated with an increase in birth-weight of 250g in males. Similarly, four adverse alleles from 2 SNPs were associated with an increase in birth-weight of 116g in females.
Genetic variants in the IGF-axis appear to play a significant role in antenatal and postnatal growth. When multiple SNPs were modelled together, large effects on fetal growth were apparent. New analytic tools utilizing the biology of entire signalling pathway would further improve our understanding of the genetic basis of antenatal and postnatal growth.

PIII-377

Longitudinal Growth of Fetal Soft Tissue in Different Ethnic Groups. Graham K. Parry. *Women's Health, Counties Manukau DHB, Auckland, New Zealand.*
To measure fetal soft tissue using 3D ultrasound in different ethnic groups.
Fetuses from four ethnic groups (Pacific Island, Maori, New Zealand European and Indian) were scanned by ultrasound at four weekly intervals from approximately 18 weeks Gestational Age. Measurements included 3D volume data sets of fetal limbs for limb circumference and volume measurements.
After delivery the newborn baby was weighed and measured (Crown Heel Length, Head Circumference and Thigh Circumference).
Birthweight was customized for maternal height, weight, parity and ethnicity.
Macrosomia was defined as >90th customized centile.
IUGR was defined as <10th customized centile
Measurements were compared with maternal Ethnicity.
Longitudinal growth of the fetal thigh circumference (as a marker of soft tissue) was measured from the 3D volume dataset.
127 women were recruited with normal ethnic related BMI. After exclusion for premature delivery, medical disease, IUGR or macrosomia there were 27 Pacific Island, 14 Maori, 26 European and 15 Indian women with no fetal abnormalities delivering at term available for analysis of the normal range. This range was used to compare measurements for fetuses that were born IUGR or macrosomic.
Birthweight was significantly different between the normal groups ($p<0.001$).
Neonatal Crown Heel length ($p=0.002$), Head Circumference ($p=0.002$) and thigh circumference ($p<0.001$) were different between the normal groups. Head Circumference/Crown Heel length ratio was not significant ($p=0.59$).
Dividing this ratio by Thigh circumference was significant ($p<0.001$)
Ultrasound review of the thigh circumference showed no significant difference between the groups until 34 weeksgestational age ($p=0.04$)
At 34 weeks fetuses macrosomic at birth showed a significantly different thigh circumference in the different ethnic groups ($p=0.05$). Fetuses that were born with IntraUterine Growth Restriction did not show a significant difference ($p=0.65$).
This lack of difference in IUGR may be because differences in a small measurement may be difficult to detect or because IUGR can be late onset in origin.
Different ethnic groups have been shown to have different fat content and different cardiovascular risk for the same BMI.
This study has shown a difference in fetal and neonatal soft tissue in different ethnic groups. This difference needs to be allowed for when determining macrosomia and IUGR.
This may be important in analysing risks for long term cardiovascular disease and diabetes.

PIII-378

BMI and Weight Gain Are Associated with Slow Intrauterine Growth, but Not with Small for Gestational Age (SGA). M.C. Roland[1], C. Friis[1], N. Voldner[1], K. Godang[2], J. Bollerslev[2,3], G. Haugen[1,3], T. Henriksen[1,3]. *[1]Obstetrics, Oslo University Hospital, Norway; [2]Endocrinology, Oslo University Hospital, Norway; [3]University of Oslo, Norway.*
SGA includes both constitutionally small newborns and those who fail to reach their genetic potential of growth.
We have categorized growth restriction according both to intrauterine growth percentiles and small for gestational age (SGA).
The aim of the study was to explore the associations between maternal characteristics and three categories of fetal growth restriction;

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(a) small for gestational age (SGA), but normal intrauterine growth
(b) slow intrauterine growth in third trimester, but normal birthweight
(c) both SGA and slow intrauterine growth
A prospective study including 1031 healthy Caucasion women. Maternal characteristics were parity, BMI, weight gain during pregnancy, maternal birthweight and mean PI (pulsatility index) in the uterine arteries.
Fetal growth was analysed by a conditional growth model. Growth was calculated for the period between weeks 30-32 and 36-38 using estimated fetal weight at weeks 30-32 as baseline. Slow fetal growth was defined as growth < 10 conditional percentile. Small for gestational age was defined as birthweight < 10 percentile for gestational age.
Associations between maternal characteristics and the categories of fetal growth restriction were explored by multinomial logistic regression, comparing the three categories of fetal growth restriction with normal fetal growth as referance.
SGA is associated with maternal birthweight (OR 0,40; 0.21,0.75, p=0,004) and PI in the uterine arteries (OR 4,36; 1.42,13.38, p=0,01).
Slow intrauterine growth is associated with BMI (OR 0,91; 0.85,0.98, p=0,013) and weight gain (OR 0,88; 0.81,0.95, p=0,002).
Being born both SGA and experiencing slow growth in third trimester is associated with BMI (OR 0.88; 0.67,0.97, p=0,005), weight gain (OR 0,79; 0.66,0.93, p=0,007), maternal birthweight (OR 0,35; 0.15,0.85, p=0,02), PI in the uterine arteries (OR 5,6; 1.34, 23.4, p=0,018) and parity (P0 vs P1 OR 18,6; 2.4, 143.0, p=0,005).
The maternal characteristics associated with three categories of fetal growth restriction differ.
For SGA the determinants are of constitutional or vascular type, whereas for slow intrauterine growth metabolic factors seem to play a role.
The small group of newborns that experience both birthweight < 10 percentile and slow growth have a range of risk factors including both constitutional, vascular and metabolic factors.

PIII-379

Voluntary Exercise in Pregnancy in the Rat Affects Axial Length but Not Body Composition in Young Adult Male Offspring. Brielle V. Rosa[1,2], Elwyn C. Firth[1,2], Hugh T. Blair[1,2], Mark H. Vickers[1,3], Patrick C.H. Morel[1,4]. *[1]National Research Centre for Growth and Development, New Zealand; [2]IVABS, Massey University, New Zealand; [3]The Liggins Institute, University of Auckland, New Zealand; [4]IFNHH, Massey University, New Zealand.*
Little is known about the long term effects of exercise during pregnancy on the health of the offspring. We previously demonstrated that voluntary exercise in the pregnant rat improves fetal growth through day 19 of pregnancy without inducing maternal stress.[1] In this study we examine the effects of maternal exercise during pregnancy on offspring postnatal growth and body composition.
Female rats were randomly divided into control and exercise groups and then mated. Rats in the exercise group were housed in raised cages requiring them to rise to an erect bipedal stance to obtain food and water throughout their pregnancy. Offspring body weight was assessed at day 1, 25, and 112. Male offspring underwent dual-energy x-ray absorptiometry (DXA) and length measurements at postnatal day 116.
Body weight did not differ between the offspring of exercised and control dams at any time point although at all assessment times females were lighter than males. DXA revealed no differences in whole body composition or bone mineral content (bmc), area, or density (bmd). Scans of the lumbar vertebrae and femur also revealed no differences between groups. Offspring of exercised rats had a shorter spine length (from base of skull to ischiatic tuberosity) than controls (p=0.02).
Although we previously found that fetuses of exercised dams were heavier at day 19 of gestation than fetuses of controls this difference was not evident on day 1 of postnatal life. Day 19 of pregnancy is after the period of organogenesis in the rat but prior to the period of rapid fetal growth. This suggests that the effects of dam exercise on fetal growth may be greatest during the period of organ formation. The differences in spine length between young adult male offspring of exercised and control dams indicate that dam exercise may affect offspring growth in postnatal life. Although DXA did not reveal differences in body composition in young adulthood there may be alterations in gene expression that would lead to more evident morphological differences as the animals age. We are currently undertaking expression analysis of genes associated with skeletal and metabolic health.
1. Rosa BV, Firth EC, Blair HT, Vickers MH, Morel PC. Am J Physiol Regul Integr Comp Physiol. 2011.

PIII-380

Maternal Age at Child Birth as Predictor of Offspring Growth and Metabolic Risk as Adults. H.P.S. Sachdev[1], S. Sinha[1], C. Osmond[2], C.H.D. Fall[2], P. Prabhakaran[3], S.K. Bhargava[4]. *[1]Sitaram Bhartia Institute of Science and Research, New Delhi, India; [2]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom; [3]Public Health Foundation of India, New Delhi, India; [4]Sunder Lal Jain Hospital, New Delhi, India.*
Young maternal age is associated with lower birth size and early childhood undernutrition, which have an inverse association with adult cardiovascular risk factors. We quantified the relation between maternal age and offspring outcomes including serial anthropometry from birth and metabolic risk factors as adults.
8,181 singleton live births to married women followed up in New Delhi in 1969-1972. Anthropometry was prospectively recorded at birth and 6-monthly until 14-21 years. Metabolic risk factors were measured in 1,526 as adults in 1998-2002 and 1100 were re-evaluated in 2006-2009; tests included grip strength and dual X-ray absorptiometry (DXA in 565). Outcomes analyzed by multivariate regression included weight and height (birth, 6 mo, 2 yr, 11 yr, adult); blood pressure, fasting and 2-h glucose, insulin resistance, cholesterol, skinfold thickness (30 years); and grip strength, lean and fat mass by DXA (36 years). Confounders adjusted for exposure of interest (maternal age at child birth) included gender, gestation, parity, maternal education, wealth, income and adult age.
Maternal age had a significant inverted U-shaped association with weight and length at birth, 6 mo and 2 yr; and height adjusted adult grip strength and lean mass (p for quadratic trend=0.02 and 0.039, respectively). There was a U-shaped relationship between fasting and 2-h glucose concentration and mother's age (p=0.016 and 0.002, respectively), which persisted even after adjustment for adult body mass index for 2-h glucose (p=0.002). The 2-h glucose unadjusted geometric mean (mg/dl) was 105 at age 26-30 years (minimum), and 114 and 112 at age up to 20 years and above 35 years, respectively. No significant relationship was documented with other adult outcomes.
Both extremes of maternal age at child birth are associated with important adverse outcomes in offsprings including lower size at birth and two years; and lower muscle strength and lean mass, and higher glucose concentration as adults. These data highlight the need to expedite socio-cultural interventions to effectively delay early child birth in low and middle income countries like India.

PIII-381

Childhood Growth, Adult BMI and Non-Alcoholic Fatty Liver Disease – The NAFLD Liver Fat Score Applied on the Helsinki Birth Cohort Study (HBCS). Samuel Sandboge[1], Clive Osmond[2], Eero Kajantie[1], Johan G. Eriksson[1,3]. *[1]Department of Chronic Disease Prevention, Diabetes Prevention Unit, National Institute for Health and Welfare, Finland; [2]MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, United Kingdom; [3]Department of General Practice and Primary Health Care, University of Helsinki, Finland, Finland.*
Intrauterine growth and subsequent growth during childhood is related to a number of adult diseases, including type 2 diabetes, coronary heart disease, and the metabolic syndrome (MetS). MetS in turn is closely associated with Non-alcoholic fatty liver disease (NAFLD) - one of the most common causes of chronic liver disease worldwide. The aim of this study was to explore the early life origins of NAFLD by applying the NAFLD liver fat score (Kotronen A *et al.* Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology. 2009;137(3):865-72) on the Helsinki Birth Cohort Study (HBCS).
The NAFLD liver fat score is calculated using five variables; the metabolic syndrome, type 2 diabetes, fS-insulin, fS-AST and AST/ALT-ratio. The optimal cut-off point, -0.640, indicated NAFLD with a sensitivity of 86% and a specificity of 71% against diagnosis by proton magnetic resonance spectroscopy in the original study population. For purposes of the current

study, we defined values greater than -0.640 as a "positive NAFLD score". We studied 1725 members of the Helsinki 1934-44 birth cohort, for whom data on both early growth as well as adult clinical measurements were available. Only individuals with a reported daily alcohol intake of < 20 g were included.

A positive NAFLD score was found in 43% of men and 24% of women. In a logistic regression model controlling for age and sex, NAFLD score was positively associated with current BMI (OR 1.33, 95% CI 1.29 to 1.38 per kg/m2, p<0.001) and negatively with BMI at two years (OR 0.82, 95% CI 0.75 to 0.91 per kg/m2, p<0.001). Those who had greater BMI at age 11 years than would have been predicted by their size at birth and BMI at two years where more likely to have a positive NAFLD-score (OR 1.23, 95% CI 1.10 to 1.37 per SD of this measure, p<0.001).

Small body size during childhood, followed by a rapid increase in BMI, seems to increase the risk of NAFLD in adulthood.

PIII-382

High Protein Content of Early Diet Programs Later Adiposity in Rats. Yasaman Shahkhalili, Mireille Moser, Corinne Ammon Zufferey, Florence Blancher, Julie Moulin, Katherine Mace. *Nutrition and Health, Nestlé Research Center, 1000 Lausanne 26, Switzerland.*

Early high protein intake is associated with higher body weight and BMI during childhood and adolescence. However, the later impact of early protein intake on body composition and blood variables in adult is not yet known. Thus the aim of this study was to compared the consequence of early protein intake at two levels of optimal and high (as fat or carbohydrate exchange) on later body composition, IGF-1 and glycemia in adult rats.

Three groups of male SD rats (n=25) were pair-fed with post-suckling diets varying in protein (casein), fat and carbohydrate as follows: 40, 10 and 50%E (HP-LF), 20, 10 and 70%E (AP-LF: protein exchange with carbohydrate), 20, 30 and 50%E (AP-MF: protein exchange with fat) from age of two to five weeks. All groups were then fed ad-libitum with a commercial chow diet (13% fat, kliba 3434) for 10 weeks, followed by a commercial high fat diet (45% fat, Kliba 2126) for 31 weeks. Food intake, body weight, body composition (NMR, Echo MRI 2004) and blood variables were measured at different ages. Glucose was measured by an enzymatic method with Cobas FARA and IGF-1 by ELISA method.

The HP-LF group relative to the other groups with an optimal protein intake (AP-LF and AP-MF) had significantly lower % body fat at age of five weeks but developed higher % body fat later at age of 174 days. Furthermore the HP-LF group showed higher plasma IGF-1 relative to both AP-LF and AP-MF groups and higher glycemia relative to the AP-LF group at the end of study: (p<0.05, in all cases). The body weight and energy intakes of all groups were similar.

The results provide the evidence for programming effect of high protein intake during early life on later adiposity and IGF-1 (as either carbohydrate or fat exchange), as well as elevated glycemia (as carbohydrate exchange) in rodents.

PIII-383

Ethnic Differences in Neonatal Body Composition in a Multiethnic Population in Oslo, Norway. The STORK-Groruddalen Study. Line Sletner[1,2,5], Britt Nakstad[2,5], Chittaranjan Yajnik[3], Kjersti Mørkrid[1,5], Siri Vangen[4], Kåre Birkeland[1,5], Anne Karen Jenum[1,5]. *[1]Oslo Diabetes Research Centre, Oslo University Hosp., Aker, Norway; [2]Dep. of Pediatrics, Akershus University Hosp., Norway; [3]Diabetes Unit, King Edward Memorial Hosp., Pune, India; [4]Dep. of Obst. and Gyn., Oslo University Hosp., Rikshospitalet, Norway; [5]Faculty of Medicine, University of Oslo, Norway.*

To describe neonatal body composition, in relation to maternal body composition, in South Asian, East Asian, Middle East and Somali neonates, (mothers mostly 1.st gen. immigrants), with Scandinavians as reference, in a multiethnic population in Oslo, Norway.

Population-based cohort study of healthy pregnant women in primary antenatal care, and *their offspring.* Inclusion from May 2008-May 2010. Maternal demographics and anthropometrics were collected by midwifes at GW 10-20. Neonatal anthropometrics were performed within 72 hours after birth. Sd-scores were calculated (neonates: sex- and gestation-specific), with the Scandinavians as reference. Mean sd-scores (with 95% CI) are presented.

823 women (59% of non-Scandinavian origin, 74% of all eligible) were included. Of 689 healthy, singleton neonates born GW ≥37 in the two study hospitals, 532 (78%, and representative) were measured with study-specific anthropometrics. All ethnic minority women were shorter, (range: East Asians: -1.88 (-2.31 to-1.44) to Somalis: -0.57(-1.06 to -0.08) compared to the Scandinavians. BMI at inclusion was lower than the Scandinavians in South Asian (-0.22 (-0.37 to -0.07)) and East Asian women and higher in Somali women (0.77 (0.05 to 1.49)). South Asian (-0.39), Middle East and Somali women had larger subscapular skinfolds.

South-Asian neonates were smaller in all body measurements, most marked for abdominal circumference (AC) (-0.89 (-1.02 to -0.72)), while length and all skinfolds were relatively preserved (though lower) (sd range: -0.28 to -0.39). East Asian neonates also had lower birthweight and AC than Scandinavian neonates, but to a lesser degree than South Asians. Middle East neonates showed just minor deviations from the Scandinavians, and in Somali neonates only AC differed (-0.69 (-1.02 to -0.35).

Although all ethnic minority women were shorter and had relatively more subcutaneous fat, neonatal body composition varied substantially between ethnic minorities. This may indicate that factors beyond maternal size and body composition influence ethnic differences in neonatal size and body composition.

PIII-384

Development of Bioelectrical Impedance Analysis Prediction Equation of Fat-Free Mass in Singapore Infants. S.E. Soh[1], M.T. Tint[1], M.A. Izzuddin[1], S.M. Saw[1], K.Y.C. Kwek[2], Y.S. Chong[1], P.D. Gluckman[3], A. Chinnadurai[1], K. Godfrey[4], L. Ward[5], F.K.P. Yap[2], Y.S. Lee[1]. *[1]National University Health System, Singapore; [2]KK Women's and Children's Hospital, Singapore; [3]Singapore Institute for Clinical Sciences, Singapore; [4]University of Southampton, United Kingdom; [5]University of Queensland, Australia.*

Predictive equations for estimating body composition (BC) using bioelectrical impedance analysis (BIA) in Asian infants are lacking. We aimed to develop an equation to predict fat-free mass (FFM) of Singapore infants, to facilitate population-based longitudinal studies of the impact of BC changes during infancy on long term metabolic outcomes.

Preliminary analyses of data from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort study were performed. FFM of infants at birth (n=157) and 4-21 days (n=149) was derived from air displacement plethysmography (PEA POD®) as the reference standard, and multifrequency BIA measurements using the Impedimed SFB7. Using data from 153 neonates (78 at birth, 75 at 4-21days, 50% males) predictive equation was derived by multiple linear regression analyses and cross-validated in the other 153 neonates. This equation was used for estimating infant FFM from single frequency BIA (Impedimed DF50) measurements at ages 3 weeks (n=189), 3 months (n=102), 6 months (n=45) and 9 months (n=20).

FFM predicted from the body mass and BIA resistance index [Length^2/R50 (cm^2/Ω)] (where R50 is the resistance at 50kHz) showed a correlation coefficient (adjusted R^2) of 0.73 and root mean square error (RMSE) of 0.11kg. From the model, the predictive equation derived from the present sample (n=313) is FFM% = (L2/R50*0.044)+(weight*0.696)+0.423. This was significantly correlated to FFM derived from PEA POD on infants from the validation group (r = 0.839, p <0.001). The 95% limits of agreement for Bland-Altman analysis is -0.309, +0.261 kg FFM. Using this equation, the mean (SD) FFM% of infants at three weeks, three months, six months and nine months are estimated as 86.4 (1.8), 81.2 (1.2), 79.7 (1.4) and 79.3 (1.6), respectively.

Our data provide a glimpse of BC changes in Singapore babies in the first nine months of life. Once validated, this predictive equation will allow for reliable and convenient BIA measurement of BC during infancy in a home visit setting. Race and gender-specific equations may be later derived as our sample size increases.

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PIII-385

Quantification of Abdominal Fat Compartments by Magnetic Resonance Imaging (MRI) in Asian Neonates. M.T. Tint[1], M.A. Izzudin[1], S.E. Soh[1], S.M. Saw[1], K. Kwek[2], Y.S. Chong[1], P. D. Gluckman[3], K. M. Godfrey[4], A. Chinnadurai[1], V. S. Rajadurai[2], P. Agarwal[2], F. Yap[2], B. Shuter[1], Y.S. Lee[1], M. V. Fortier[2]. *[1]National University of Singapore, Singapore; [2]KK Women's and Children's Hospital, Singapore; [3]Singapore Institute of Clinical Sciences, Singapore; [4]University of Southampton, United Kingdom.*

Body composition at birth reflects in-utero developmental influences and may determine subsequent metabolic phenotype. Quantity and distribution of adiposity in neonates can be studied using MRI. However, image analysis algorithms distinguishing specific fat compartments are lacking. The aim is to develop a comprehensive analysis program for segmentation and quantification of abdominal fat in Asian neonates in the Growing Up in Singapore Towards healthy Outcomes(GUSTO) study.

We analysed MRI data(T1-weighted; 1.5T scanner) collected within 21days of delivery from an initial group of 95 neonates≥34 weeks gestation and weight≥2 kg (Chinese[15Male/23Female], Malay[23M/18F] and Indian[9M/7F]). Abdominal fat tissue from the level of diaphragm to the sacrum was divided into superficial subcutaneous(ASSC), deep subcutaneous(ADSC) and internal(AI). Volumes of fat tissue(ml) were determined using an in-house MATLAB package comprising automatic processing(watershed transform of local standard deviation, initial identification of ASSC and AI compartments) and manual routines.

Initial observations suggested that gender differences in abdominal fat distribution may vary between ethnic groups. Chinese females had a greater ASSC than Chinese males(mean 83.8vs98.8ml; p=0.03), with similar ADSC and AI. For all three compartments there were trends for Malay female infants to have greater fat than Malay males, and for Indian male infants to have greater fat than Indian females but these differences were not statistically significant($p>0.05$). Preliminary analyses showed weak trends for Indian infants to have greater ASSC and ADSC(94.3&14.4ml) than Chinese(92.9&14.0ml) and Malay(89.5&13.6ml) infants; however, Indian infants had less AI(24.6ml) than Chinese(27.1ml) and Malay(24.3ml) infants(all $p>0.05$).

MRI can be used to measure specific fat depots in birth cohort studies. Analysis of a larger sample size in the GUSTO cohort is in progress and may provide valuable insights into developmental influences on early life fat distribution, and their role in ethnic differences in metabolic risk observed later in life. Our in-house analysis package may be a valuable research tool.

PIII-386

Effect of Birthweight and Weight Gain during Infancy on Blood Pressure in Young People. Claudia Cinthya Urquidi B.[1], Patricia Bustos[2], Hugo Amigo[2]. *[1]Programa de Doctorado de Salud Pública, Escuela de Salud Pública. Universidad de Chile, Chile; [2]Departamento de Nutrición, Facultad de Medicina. Universidad de Chile, Chile.*

To evaluate whether weight gain (WG) during infancy is conditioned by weight at birth, and to separate the effect of birthweight (BW) and WG on blood pressure in young people born in Valparaíso, a region of Chile.

The study included 1,231 subjects aged 22-28 years old who were randomly selected from a sampling frame of 3,096 newborns between 1 January 1974 and 31 December 1978 in the maternity hospital in Limache (a small agricultural town in the Valparaíso Region). Blood pressure was recorded twice by two trained nurses; because a high concordance between measurements a simple blood pressure mean was used. Weight at birth and at six, nine and 12 months of age were obtained from clinical records and transformed into Z scores according to the reference values established by the WHO. Since missing anthropometric values were at random, mixed-effect models with random intercept and random slope were fitted to estimate for each subject. WG was calculated as the difference of the random coefficients. A partial correlation coefficient between the intercept (BW) and slope (WG trajectory) was also calculated through the mixed model. Linear regression models were fitted to estimate the effect of BW and WG. The joint effect of BW and WG was estimated through an interaction term. All the analysis was done by adjusting by mother´s schooling, breastfeeding and gender. A p value <0.05 were considered as significant.

45% of the sample was men. The median systolic and diastolic blood pressure (SBS, DBP) were 133.5 (IR=18) and 72 mmHg (IR=11.5), respectively. The partial correlation between BW and WG was -0.3 (95% CI -0.2 to -0.4). There was an inverse correlation between BW and SBP (β=0.61, p=0.035), but this significance disappeared after controlling for confounders. There was a significant and positively adjusted association between WG from six to nine months old for both SBP and DBP (β=1.96, p=0.042; β=2.5, p=0.014). The interaction term between BW and WG was not significant.

Weight gain during infancy is moderately conditioned by weight at birth. There was an independent and positive effect of weight gain during infancy on systolic and diastolic blood pressure in this population.

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PIII-387

Body Composition of the Newborn Predicts Future Diabetes Risk in Both Parents; Mysore Parthenon Study. S.R. Veena[1], G.V. Krishnaveni[1], J.C. Hill[2], M.S. Rajesh[1], C.H.D. Fall[2]. *[1]Epidemiology Research Unit, Holdsworth Memorial Hospital, India; [2]MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, United Kingdom.*

It has been suggested that a baby's birthweight (BW) is a "window" on the health of the parents. We aimed to examine detailed body measurements of babies at birth as predictors of diabetes in the parents nine years later.

The baby's weight (BW), crown-heel length (CHL), crown-rump length (CRL), leg length (LL), triceps and subscapular skinfolds (TR, SS), and circumferences of the head (HC), abdomen (AC) and mid-upper-arm (MUAC) were measured at birth in a cohort of 622 babies born to non-diabetic mothers in Holdsworth Memorial Hospital, Mysore, India. Nine years later an oral glucose tolerance test (WHO protocol) was performed in 473 mothers (76%) and 401 fathers (64%) of these children.

65 (16%) fathers and 23 (5%) mothers had diabetes. The children's BW showed a linear inverse association with the prevalence of diabetes (DM) among fathers (prevalence=20%, 19% and 9% in fathers of babies in low, middle and high tertiles of BW respectively; Odds Ratio 0.4 per kg of BW, 95% CI: 0.2, 0.8; p=0.004).

There were similar linear inverse associations of newborn CHL, CRL, TR, SS and MUAC with DM in the fathers ($p<0.05$, all).

In contrast, the children's BW showed a U-shaped association with DM prevalence in mothers (7%, 3% and 5% across low, middle and high BW tertiles; p for quadratic association=0.01). There were similar U-shaped associations of newborn TR, SS, AC and MUAC with DM in the mother ($p<0.05$, all). Newborn CHL showed, as in fathers, a linear inverse association with DM prevalence in mothers (p=0.01).

All the associations described remained statistically significant after adjusting for the child's sex, gestational age, the parents' age and adiposity (BMI and skinfolds), and maternal glucose concentrations during pregnancy.

Smallness in all body components at birth except LL and HC predicts an increased risk of later diabetes in both parents; this suggests a genetic or epigenetic link between diabetes risk in either parent and reduced fetal growth in their children.

Additionally, higher BW and greater newborn adiposity predict an increased risk of maternal diabetes; this suggests either that pre-diabetic metabolic changes in the mother during pregnancy (other than her glucose concentrations) increase fetal adiposity, or that fetal adiposity induces maternal diabetes.

PIII-388

Markers of Maternal Perinatal Nutrition Are Associated with Infant Weight Gain during the First Six Postnatal Months in Rhesus Monkeys. Jennifer A. Villar[1], Natalie A. Brutto[2], Melinda Higgins[3], Milton R. Brown[1], Mark E. Wilson[2], Andrew Muir[1]. *[1]Department of Pediatrics, Emory University, GA, USA; [2]Yerkes National Primate Research Center, Emory University, GA, USA; [3]School of Nursing, Emory University, GA, USA.*

Programming of early metabolism may influence the risk of later obesity. Since some rhesus monkeys spontaneously develop obesity under normal living conditions, they can be used to study maternal affects on infant growth. They have singleton pregnancies lasting 165 days and infants wean near six months (mos). Anthropometrics, body composition, and circulating

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leptin concentrations of mothers and their babies were studied from mid-gestation until infant weaning. We hypothesized that maternal nutrition would influence early infant growth.

18 pregnant rhesus monkeys were randomly selected. Anthropometric measures and DEXA scans were obtained on dams at gestational day 100 & 150 and 1 & 5 mos post-partum (pp). Dams and their infants were weighed near pp days 2, 7, 14, 28 and then monthly. Serum leptin 1 mo pp was measured by RIA.

Among healthy neonates, growth in the first six mos fits a second order quadratic relationship. After accounting for the association of maternal midgestational lean body mass with infant birth weight (r^2=0.604, p<0.001), post natal growth was significantly related to pp maternal fat accretion and circulating leptin (p=0.002). Circulating leptin concentrations in the mother predicted infant weight gain better than did infant leptin levels. Both maternal circulating leptin at 1 mo pp and maternal fat accretion between late gestation and 1 mo pp (Δfat) predicted infant weight gain for over the first six months. Higher leptin and Δfat predicted faster initial weight gain and an earlier deceleration. Infant weight gain was not significantly associated with maternal Δfat between 1& 5 months pp, age, parity, social rank, or infant gender.

Whereas maternal size influenced fetal growth, weight gain in early infancy was related to markers of maternal nutrition during late gestation and the first pp month. Consistent with early metabolic programming, maternal fat accretion between one and five months pp did not correlate with the rate of infant weight gain.

PIII-389

Genetic Variants in Adult Obesity Genes Are Associated with Childhood Growth. N. M. Warrington[1], J. A. Marsh[1], J. P. Newnham[1], L. J. Beilin[2], S. J. Lye[3], L. Briollais[3], C. E. Pennell[1]. *[1]School of Women's and Infants' Health, The University of Western Australia, Australia; [2]School of Medicine and Pharmacology, The University of Western Australia, Australia; [3]Samuel Lunenfeld Research Institute, University of Toronto, Canada.*

An individual's susceptibility to obesity is a result of interactions between genetics, lifestyle and the environment. Individual growth curves throughout childhood reflect their environment (previous and current) and their genetic predisposition. A number of genetic variants have been reported to be associated with adult obesity; however, their influence on growth during childhood is unknown. Aim: To examine the of effect genetic variants associated with adult obesity on growth throughout childhood.

Eight measures of BMI were collected on individuals from the Western Australian Pregnancy (Raine) cohort (N=1,489) from 1-17 years. Growth characteristics were defined for overall size, growth velocity and timing of adiposity rebound. Sixteen single nucleotide polymorphisms (SNPs) have been shown previously to be associated with adult obesity. Multivariate linear regression, stratified by gender, was used to evaluate epidemiological and genetic associations with each of the growth characteristics.

Birth weight, maternal smoking during pregnancy, weight gain in the first year of life, socio-economic status, duration of breastfeeding and maternal gestational weight gain were associated with the growth characteristics throughout childhood. Of the 16 SNPS, the SNPs from the FTO, MC4R, MTCH2 and SH2B1 genes were associated with an increase in overall size throughout childhood in males accounting for 2.2% of the variation between individuals. Similarly, two SNPs from the FTO and SEC16B genes were associated with increased size in females accounting for 0.9% of the variation. The FTO and MC4R SNPs in males and the SEC16B SNP in females were also associated with an earlier adiposity rebound, accounting for 4.9% and 0.8% of the variation respectively. Only one SNP from the SH2B1 gene in males was associated with lower growth velocity.

This study provides evidence that a small number of the adult obesity genes begin having an effect early in life. However, the variants which affect early life growth differ by gender. Further research is required in this area, particularly in larger cohort studies. This may offer the opportunity to identify individuals early in life who are at increased risk of developing obesity for targeted interventions.

PIII-390

Etiological Subgroups of Small-for-Gestational-Age: Differential Childhood Growth Trajectories. Xiaozhong Wen. *Obesity Prevention Program, Dept of Population Medicine, Harvard Pilgrim Health Care Institute and Harvard Medical School, MA, USA.*

Newborns of small-for-gestational-age (SGA) are a heterogonous group, regarding both causes and long-term outcomes. Subgroups of SGA newborns due to different factors may have different postnatal growth trajectories. We examined childhood growth trajectories of SGA subgroups associated with different maternal factors.

We analyzed data of 1,220 SGA singletons (birth weight < 10th percentile by sex and gestational age) in the Collaborative Prenatal Project,a national pre-birth cohort in USA. Based on three well-established risk factors, SGA newborns were classified into four subgroups: 1)with maternal smoking during pregnancy only (n=206), 2)with low maternal pre-pregnancy body mass index (BMI) only (n=382), 3)with low pregnancy weight gain only (n=54), and 4)with none of the three maternal factors (n=578). Children's height/length and weight were measured at birth, 4 m, 1 y, 4 y, and 7 y. We used piecewise linear mixed models to fit and compare childhood growth trajectories across four subgroups, adjusting for family socio-economic status (SES) percentile; maternal age, race, marital status, parity; and the child's sex.

The four subgroups of SGA children were comparable in sex, gestational age, and family SES. Overall, they had similar height/length trajectories from birth until 4 y. However, at seven year, the subgroup of SGA children associated with low maternal pre-pregnancy BMI were significantly shorter than children in other three subgroups. The four subgroups of SGA children were comparable in weight at birth and 4 m, after which their differences in weight became significant and amplified with age: the subgroup associated with maternal smoking during pregnancy > the subgroup with none of the three maternal factors > the subgroup associated with low pregnancy weight gain > the subgroup associated with low pre-pregnancy BMI. Similar subgroup-level differences were also observed in their BMI trajectories; but the gap in BMI means increased with age between birth and 1 y, narrowed down at 4 y, and then exaggerated again at 7 y. Sex-stratified analysis did not yield substantially different results from the total sample.

Etiological subgroups of SGA children have differential childhood growth trajectories (especially weight and BMI). SGA children associated with maternal smoking have the highest attainments in weight and BMI after 4 m, whereas SGA children associated with low pre-pregnancy BMI tend to be shorter and thinner at 7 y.

PIII-391

Children Born Preterm Have Reduced Long Term Depression (LTD)-Like Neuroplasticity. Julia B. Pitcher, Alysha M. Riley, Michael C. Ridding. *Robinson Institute, School of Paediatrics & Reproductive Health, The University of Adelaide, Australia.*

Neuroplasticity is the brain's ability to change the strength of its synaptic connections in response to activity and experiences. It is believed to be the physiological basis for learning and memory. Preterm children have alterations in cortical development, functional connectivity and patterns of neural activation in response to incoming stimuli, suggesting that their capacity for neuroplasticity is also reduced and may contribute to their common difficulties with learning and memory. We hypothesized that, compared with their term-born peers, children born <37 weeks completed gestation (wks GA) have a reduced response to a non-invasive neuroplasticity induction intervention designed to induce a short-term LTD-like (i.e. inhibitory) change in motor cortex (M1) excitability.

25 children (15 females) aged 12-15 years (13.67 ± 0.48 years) participated; Term born (37-41 wks GA) n=6, Late preterm (33-36 wks GA) n=9 and Early preterm (25-32 wks GA) n=9. Continuous theta burst stimulation (cTBS) was applied to the M1 to induce LTD-like neuroplasticity. To assess changes in M1 excitability (an indicator of neuroplasticity), single pulse transcranial magnetic brain stimulation was used to record motor evoked potentials (MEPs) from a hand muscle before and for 60 min following cTBS.

Term born children showed robust M1 inhibition immediately following cTBS and this persisted for at least 60 min. The depth and persistence of the inhibition in this group was greater than previously consistently recorded in adult subjects. In comparison, inhibition in both preterm groups was

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significantly less than term born children and returned to baseline within 40 min of cTBS ceasing. GA correlated negatively with the mean MEP inhibition following cTBS (i.e. the least inhibition was evoked in the most preterm children).

These preliminary findings provide the first physiological evidence of reduced neuroplasticity in preterm children. While different types of neuroplasticity induction (i.e. LTP-like, behavioural) are yet to be assessed, these results demonstrate that even modest levels of prematurity are associated with significant impairments that persist at least into early adolescence. The underlying mechanisms are not yet clear, but may include synapse specific dysfunction and/or altered cortisol secretion patterns which are known to influence neuroplasticity.

PIII-392

Preterm Birth and Lipid Profile in Adolescence. Marika Sipola-Leppänen[1], Marjaana Tikanmäki[1], Marja Vääräsmäki[1,2], Petteri Hovi[1,3], Anneli Pouta[1,2], Aimo Ruokonen[4], Anna-Liisa Hartikainen[2], Marjo-Riitta Järvelin[1,5,6], Eero Kajantie[1,3]. *[1]National Institute for Health and Welfare, Oulu and Helsinki, Finland; [2]Department of Obstetrics and Gynaecology, Oulu University Hospital, Oulu, Finland; [3]Hospital for Children and Adolescents, Helsinki University Central Hospital, Helsinki, Finland; [4]Department of Clinical Chemistry, Oulu University Hospital, Oulu, Finland; [5]Imperial College, London, United Kingdom; [6]Institute of Health Sciences, University of Oulu, Oulu, Finland.*

Preterm birth is associated with increased levels of cardiometabolic risk factors later in life, including high blood pressure and impaired glucose regulation. We studied the association of preterm birth with lipid profile in adolescence.

The subjects come from the population-based Northern Finland Birth Cohort 1986, which is a prospective and longitudinal one-year birth cohort representing an unselected population. At the mean age of 16.0 years, 6226 of the 9479 cohort members attended an clinical examination which included measurements of serum fasting total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), apolipoprotein A1 (ApoA1), and B (ApoB) concentrations.

Among boys, adolescents born before 34 weeks of gestation (n=30) had 7.6% (95% CI 1.0 to 14.7%) higher TC, 14.1% (4.4 to 24.9%) higher mean LDL-C, and 13.6% (4.4 to 23.6%) higher ApoB than term-born controls. Among those born between 34+0 and 36+6 weeks+days (late preterm; n=129), the only statistically significant difference in was in TG, which was 12.1% (95% CI 3.9 to 20.8) higher compared to term-born controls. HDL-C and ApoA1 were similar between groups. Among girls, preterm birth was unrelated to lipid profiles. Interaction terms between the effects of birth <34 wk of gestation and sex were statistically significant for LDL-C (p=0.02) and ApoB (p=0.03) levels.

Adolescent boys born preterm before 34 weeks of gestation have higher concentrations of LDL-C and its primary component ApoB than boys born at term. This may contribute to an increased risk of cardiovascular disease later in life.

PIII-393

Autism- and Asperger-Related Traits in Young Adults Born Preterm: The Helsinki Study of Very Low Birth Weight Adults. Sonja Strang-Karlsson[1,2], Riikka Pyhälä[1,3], Katri Räikkönen[3], Anu-Katriina Pesonen[3], Jari Lahti[3], Petteri Hovi[1,2], Kati Heinonen[3], Anna-Liisa Järvenpää[2], Johan G. Eriksson[1,4], Sture Andersson[2], Eero Kajantie[1,2]. *[1]Diabetes Prevention Unit, National Institute for Health and Welfare, Helsinki, Finland; [2]Children's Hospital, Helsinki University Central Hospital, Helsinki, Finland; [3]Institute of Behavioural Sciences, University of Helsinki, Helsinki, Finland; [4]Department of General Practice and Primary Health Care, University of Helsinki, Helsinki, Finland.*

Recent studies suggest an increased risk of autism spectrum conditions in children and adults born preterm. We studied autism-related traits and empathy in unimpaired adults born prematurely at very low birth weight (VLBW; < 1500 g), as compared with controls born at term.

110 VLBW and 104 control adults, aged 21 to 27 years, completed two self-rating instruments for measuring traits related in particular to high-functioning autism: the Autism-Spectrum Quotient (AQ; Baron-Cohen *et al.*, 2001) yielding five subscales (social skills; attention switching; attention to detail; communication; imagination) and the Empathy Quotient (EQ; Baron-Cohen & Wheelwright, 2004) giving one total score. All subjects had attended mainstream schools. Mean birth weights (SD) in the VLBW and control groups were 1131 (216) and 3612 (490) g, and mean gestational ages were 29.3 (2.4) and 40.1 (1.1) weeks.

While there were no statistically significant group differences in AQ or EQ total scores, the groups differed in a number of AQ subscales, each representing a specific trait: VLBW adults reported poorer communication skills than controls (p=0.02), and among women, also poorer imagination. Conversely, VLBW adults reported a lower degree of attention to details, a trait characteristic of autism (p=0.02). Adjustments for sex, age, and parental education had little effect on the results. No subject reported having been diagnosed autism or Asperger syndrome.

Although we found no differences in the total scores, high-functioning VLBW adults differed from those born at term with regard to a number of specific autism-related traits.

PIII-394

Altered Micronutrients in Women Delivering Preterm. Deepali P. Sundrani, Madhavi V. Dhobale, Preeti M. Chavan, Hemlata R. Yadav, Sadhana R. Joshi. *Department of Nutritional Medicine, Interactive Research School for Health Affairs (IRSHA), Maharashtra, India.*

Maternal nutrition is an important determinant of duration of gestation and fetal growth thereby influencing pregnancy outcome. Micronutrients such as folic acid and vitamin B_{12} are involved in one carbon metabolism that underlies intrauterine programming of adult diseases. The present study was undertaken to study the levels of folate, vitamin B_{12} and resultant homocysteine in mothers delivering preterm.

76 women delivering at term (Control group), 70 women delivering preterm (PT group) and 48 women with pre-eclampsia delivering preterm (PT-PE group) with singleton pregnancy were recruited for the study from Bharati Hospital Pune, India. The levels of folate, vitamin B_{12} and homocysteine were measured by the fluorescence polarization immunoassay.

Plasma folate levels were significantly reduced in PT group (8.01 ± 5.15 ng/ml) ($p<0.05$) while there was no difference in the PT-PE group (9.2 ± 5.82 ng/ml) as compared to control (9.29 ± 4.8 ng/ml). Plasma vitamin B_{12} levels were significantly higher in both PT (204.7 ± 109.9 pg/ml) ($p<0.01$) and PT-PE (205.0 ± 129.3 pg/ml) ($p<0.05$) as compared to control group (164.6 ± 94.9 pg/ml). Homocysteine concentrations were significantly increased in PT (12.2 ± 4.4 μmol/l) ($p<0.05$) and PT-PE (15.3 ± 6.7 μmol/l) ($p<0.01$) groups as compared with control group (10.7 ± 8.7 μmol/l). Further PT-PE ($p<0.05$) showed significantly increased ($p<0.05$) levels of homocysteine as compared to PT group. There was a negative association of plasma homocysteine concentration ($r= - 0.306$; $p=0.03$; n=46) with birth weight only in the PT group. However, such an association was not seen in the PT-PE group.

This is the first report of reduced folic acid, increased vitamin B_{12} and homocysteine concentrations in women delivering preterm babies (PT group). Further studies need to be undertaken to understand the role of these micronutrients which are involved in one carbon metabolism and play an important role in epigenetic regulation of vital genes involved in fetal programming of adult diseases.

PIII-395

Reduced Levels of Placental Long Chain Polyunsaturated Fatty Acids in Preterm Deliveries. Nisha S. Wadhwani[1], Madhavi V. Dhobale[1], Savita S. Mehendale[2], Hemlata R. Pisal[1], Sadhana R. Joshi[1]. *[1]Nutritional Medicine, Interactive Research School for Health Affairs (IRSHA), Maharashtra, India; [2]Obstetrics and Gynaecology, Bharati Medical College and Hospital, Maharashtra, India.*

Placental delivery of long chain polyunsaturated fatty acids (LCPUFA) (constituents of the cell membrane and precursors of prostaglandins) is essential for the optimal development of the central nervous system of the fetus. Placenta in preterm birth is suggested to provide clues to predicting the risk of individuals developing chronic diseases in later life. The present study examines the levels of LCPUFA and their association with placental weight in women delivering preterm and at term.

Women delivering preterm (n=58) and at term (n=44) were recruited for the study from Bharati Hospital Pune, India. Placental fatty acid analysis was performed by using Perkin Elmer gas chromatograph.

Total saturated fatty acids (46.07 ± 11.28 vs 38.38 ± 5.08 g/100g fatty acids) were higher ($p<0.01$) and monounsaturated fatty acids (8.83 ± 2.26 vs 9.78 ± 2.16 g/100g fatty acids) were lower ($p<0.05$) in case of preterm deliveries as compared to term deliveries. Docosahexaenoic acid (DHA) (2.05 ± 0.97 vs 3.19 ± 0.94 g/100g fatty acids) and arachidonic acid (AA) (19.51 ± 6.15 vs 22.44 ± 3.43 g/100g fatty acids) levels were lower ($p<0.01$) while levels ALA (0.38 ± 0.36 vs 0.19 ± 0.15 g/100g fatty acids) and EPA (0.32 ± 0.29 vs 0.11 ± 0.16 g/100g fatty acids) were higher in women delivering preterm as compared to term deliveries. There was a positive association of placental DHA with placental weight in preterm group ($r = 0.413$, $p = 0.036$, n= 45).

Higher levels of ALA and EPA and lower levels of DHA in the preterm group suggest that ALA is not completely metabolized to DHA. This may be due to the reduced Δ5-desaturase enzyme activity. The reduced levels of AA in our study may be as a result of increased oxidative stress in preterm pregnancies. Our findings suggest that fetal growth may possibly be hampered due to composition of placental fatty acids and their transfer via placenta during pregnancy Micronutrients like folic acid, vitamin B12 and LCPUFA interact in the one carbon cycle. We have recently reported gestation-dependant changes in human placental global DNA methylation levels. In view of this, our results indicating reduced levels of placental LCPUFA may have implications for fetal programming of adult diseases.

PIII-396

Antenatal Steroids and Blood Pressure in Adolescents Born Preterm. Lisa K. Washburn[1], Patricia A. Nixon[1,2], T. Michael O'Shea[1]. *[1]Pediatrics, Wake Forest School of Medicine, Winston-Salem, NC, USA; [2]Health and Exercise Science, Wake Forest University, Winston-Salem, NC, USA.*

The objective of this study was to evaluate the relationship of antenatal steroid (ANS) exposure on blood pressure (BP) in adolescents who were born prematurely with very low birth weight (VLBW; ≤ 1500g).

We studied 188 fourteen-year-olds born 1992-96 with VLBW at a single medical center; 50% of them were exposed to ANS. Auscultatory BP, height, and weight were measured in triplicate on three separate visits. BP response to cold pressor test (bag of ice water placed on forehead for one minute) was assessed using an automated oscillometric device. Aerobic fitness was defined as peak oxygen uptake ($V0_{2\ peak}$) obtained by maximal exercise testing on a cycle ergometer. We made triplicate measurements of triceps and subscapular skinfold thicknesses with Lange calipers, and of abdominal circumference with a measuring tape. Percent body fat was determined by dual energy X-ray absorptiometry. Perinatal data were extracted from medical record review and existing databases. Regression analysis was used to adjust for birth weight z score, maternal hypertension, race, gender, peak oxygen uptake, height, and subscapular skinfold thickness.

Adolescents exposed to ANS were more likely to be white (68% v. 39%), born of pregnancies complicated by maternal hypertension (43% v. 29%), and have lower birth weight z scores (-0.39 v. -0.29). There were no group differences in gestational age (mean = 28 weeks) or birth weight (mean = 1047 grams). At age 14, ANS-exposed individuals were taller (height z score -0.20 v. -0.57), more fit ($V0_{2peak}$ 40 v. 36 ml/kg/min), and had lower subscapular skinfold thickness (15 v. 18 mm), but there were no group differences in other anthropometric measures (weight, body mass index, triceps skinfold thickness, abdominal circumference, percent body fat). In both unadjusted analyses as well as analyses that adjusted for confounders and mediators, we found no differences in BP (105/61 v. 105/61 mmHg), systolic BP response (delta 15 v 14 mmHg) or diastolic BP response (delta 7 v. 8 mmHg) to cold pressor test.

ANS exposure is not associated with BP or BP response to cold pressor test in this cohort of adolescents born prematurely with VLBW but is associated with improved fitness, decreased subscapular fat distribution, and greater height at 14 years of age.

PIII-397

Calcium Supplementation Prevents Obesity, Hyperleptinemia and Hyperglycemia in Adult Rats Programmed by Early Weaning. Elaine Oliveira, Patricia C. Lisboa, Magna C.F. Passos, Jessica L. Nobre, Natália S. Lima, Juliana G. Franco, José F. Nogueira-Neto, Egberto G. Moura. *Physiological Sciences Department, State University of Rio de Janeiro, Rio de janeiro, Brazil.*

Obesity is a global epidemic and calcium therapy may have anti-obesity effects. Since early weaning leads to obesity, hyperleptinemia and insulin resistance, we studied the possible effect of dietary calcium supplementation on these endocrine dysfunctions in this experimental model.

Lactating rats were separated in: EW (early weaning, n=10)- dams were involved with a bandage to interrupt the lactation in the last three days of lactation, and C (control, n=10)- dams whose pups had free access to milk during all lactation (21 days). At 120 days-old, EW and C offspring were subdivided into four groups (10 animals, one from each litter): 1) C- standard diet; 2) CCa- calcium supplementation (10g of calcium carbonate/kg of rat chow); 3) EW- standard diet, and 4) EWCa- calcium supplementation. Rats were killed at 180 days. All significant data were $p<0.05$.

Adult EW offspring displayed hyperphagia (28%), higher body weight (9%) and adiposity (77%), hyperleptinemia (2 fold-increase), hypertriglyceridemia (64%), hyperglycemia (16%), higher insulin resistance index (38%) and lower adiponectinemia/adipose tissue ratio (44%). In addition, they showed lower hypothalamic JAK-2 and pSTAT-3 protein contents (36% and 34%, respectively), suggesting leptin resistance. Two months of dietary calcium supplementation normalized these disorders in EW rats. EW group had no change of serum insulin, T4 or T3 and calcium treatment did not alter these hormones.

We reinforced that early weaning leads to late development of some components of metabolic syndrome and leptin resistance. Dietary calcium supplementation seems to protect against the endocrine-metabolic disorders development in EW offspring.

PIII-398

Reversing the Effects of Maternal High-Fat, High-Sugar Feeding on Offspring Food Preferences and Fat Deposition. Zhi Yi Ong[1], Beverly S. Muhlhausler[1,2]. *[1]Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia; [2]FOODplus Research Centre, School of Agriculture, Food and Wine, University of Adelaide, Adelaide, Australia.*

We and others have previously shown that maternal intake of high-fat, high-sugar diets during pregnancy and lactation alters the development of the central reward pathway of the offspring and programs a preference towards high-fat foods after birth. This study aims to investigate whether the introduction of a low-fat diet after weaning can reverse the effects of maternal high-fat, high-sugar diet on offspring food preferences and body fat deposition.

Rat dams were either fed standard rat chow (control, n=10) or a cafeteria 'junk food' diet (JF, n=10) during pregnancy and lactation. From weaning, all offspring were given free access to control chow. At six weeks and three months of age, one male and one female offspring from each litter were given free access to both the control and cafeteria diet for three weeks and food and macronutrient preferences determined. These offspring were killed at three months and six months of age respectively and the weight of all fat depots collected for determination of total and percentage body fat mass.

At six weeks of age, the percentage of dietary energy derived from carbohydrate was higher (48.6±1% vs 43.2±1.4%; $p<0.01$) and percentage derived from fat was lower (33.7±1.3% vs 39.8±1.6%; $p<0.01$) in female JF offspring compared to controls. At three months of age, this was reversed and fat intake was higher (45.5±1.9% vs 40.5±1.4% $p<0.05$) and carbohydrate intake lower (42.0±1.7% vs 46.5±1.2%; $p<0.05$) in the female JF offspring. These differences were not present in males. There was no difference in total body fat between groups at six weeks and six months of age. However, total body fat was higher in the JF offspring at three weeks (Control: 5.9±0.2%, JF: 8.7±0.6%; $p<0.01$) and three months of age (Control: 11.0±0.6%, JF: 13.8±0.6%; $p<0.01$) in both males and females.

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A low-fat diet after weaning resulted in lower fat intake in female offspring of JF dams at six weeks, but not three months of age. Importantly, introducing a low-fat diet after weaning resulted in a reduced body fat mass before, but not after, transient introduction of a palatable diet. These findings suggest potential windows of opportunity for intervention.

PIII-399

Reduced n-6 Polyunsaturated Fatty Acids during Postnatal Development Affect White Adipose Tissue Development and Prevent Excessive Fat Deposition in Adulthood. Annemarie Oosting[1], Diane Kegler[1], Bert J.M. van de Heijning[1], Eline M. van der Beek[2]. *[1]Danone Research - Centre for Specialised Nutrition, Netherlands; [2]Danone Research, Singapore.*

Compared to lean counterparts, obese adults have a higher number of adipocytes from early age onward suggesting that preadipocyte proliferation and differentiation during early development is a major determinant of fat mass in adulthood[1]. The reported shift towards increased dietary n6 and decreased dietary n3 fatty acids (FA) intake could underlie the increasing prevalence of obesity, since dietary FA directly affect adipose tissue development[2]. We previously showed that increased levels of n3 LCP in the postnatal diet prevent excessive fat accumulation in adult mice[3]. The objective of the present study was to investigate whether reducing n6 PUFA during postnatal life has sustained effects on development of white adipose tissue (WAT) and adult body composition.

Male C57Bl/6j mice were subjected to either a control (CTR) or a low n6 PUFA (Low LA) diet, in which linoleic acid (LA) content was reduced by 50% from postnatal day (PN) two to 42. Subsequently, mice of both experimental groups were switched to a Western style diet (WSD) until dissection on PN98. Body composition was monitored by dual x-ray absorptiometry at PN42, 70 and 98. In a separate study, male Wistar Unilever rats were subjected to the same experimental protocol. After dissection at PN98, inguinal and retroperitoneal fat depots were collected and used to determine adipocyte number and size distribution.

Body weight and body composition of Low LA and CTR mice were similar at PN42. However, during WSD challenge, fat accumulation was reduced by 27% ($p< 0.001$) and lean body mass gain was moderately higher (PN70; $p< 0.05$) in mice postnatally fed Low LA.

In adult rats, lowering LA intake during postnatal development resulted in a shift in cell size distribution towards increased number of large cells. Adipocyte number did not differ significantly between both groups.

Reduction of n6 PUFA intake during early life reduced adipocyte size and prevented fat accumulation during adolescence and adulthood. This study has shown that fat quality of neonatal nutrition plays an important role in early adipocyte development and might program adult body composition and metabolic homeostasis.

1) Spalding *et al.*, Nature, 2008. 45(7196): p783-7
2) G. Ailhaud *et al.*, Prog Lipid Res., 45: 203-236, 2006
3) Oosting *et al.*, 2010, Pediatric Research 68(6): 494-499

PIII-400

Prolonged Non-Nutritive Sucking Habits: Associated Factors in Two Brazilian Cities. Marcela M. Nader[1], Felipe P. Figueiredo[1], Marco C. Barbieri[1], Viviane C. Cardoso[1], Heloisa Bettiol[1], Antonio A.M. Silva[2]. *[1]departament of pediatrics, Faculty of Medicine of Ribeirao Preto, SP, Brazil; [2]public health, Federal University of Manharao, MA, Brazil.*

To assess the association between prolonged non-nutritive sucking habits (finger and pacifier sucking) and variables concerning the mother and the child at birth and at school age among children from cohort studies in two Brazilian cities.

A longitudinal study on children from cohorts born in Ribeirao Preto (RP), SP in 1994 and in São Luis (SL), MA in1997/98 and reevaluated at school age in 2004/05. The birth data considered were birth weight, sex, and gestational age and the maternal data were age, schooling, marital status, smoking during pregnancy, parity, and working outside the home. Sociodemographic information was obtained at school age (i.e., type of feeding at the beginning of life and occurrence and duration of the sucking habit). Non-nutritive sucking habits were considered to be prolonged when maintained starting at 36 months of life. Crude and adjusted prevalence ratios (PR) of the habits were estimated by Poisson regression.

The prevalence of the habits was 47.6% in RP and 19,6% in SL. Birth weight and the remaining birth variables were not associated with the habits in RP; in SL, children of mothers living in consensual union and girls presented a higher prevalence of the habits (PR 1.50, 95%CI 1.01-2.22; PR 1.41, 95%CI 1.03-1.93, respectively). Bottle feeding was a risk factor in both cities (PR 5.62, 95%CI 2.58-12.25 in RP; PR 2.43, 95%CI 1.48-4.00 in SL). In SL, sleeping with a bottle (PR 1.44, 95%CI 1.04-2.00) and not being breast-fed were risk factors (PR 1.66, 95%CI 1.09-2.53).

Birth weight was not associated with the habits, whereas factors related to feeding were associated in both cities. Female sex and social factors such as maternal marital status were associated with the habits only in SL.

PIII-401

The Childhood Rural-to-Urban Migration and Its Effect on Obesity-Adiposity and Cardiovascular Risk Factor. S. Shukla[1], S. Chougule[1], A. Bhalerao[1], T. Deokar[1], V. Solat[1], L. Ramdas[1], C. H.D. Fall[2], C. Yajnik[1]. *[1]Diabetes Unit, KEM Hospital Research Centre, Pune, Maharashtra, India; [2]MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom.*

Rural to urban migration is considered a major risk factor for the escalating epidemic of obesity-adiposity, diabetes and CVD in India. There are very few studies to assess the influence of childhood migration on these risk factors. Pune Maternal Nutrition Study- a rural birth cohort study provides a unique opportunity to study these phenomenon.

We hypothesized that childhood migration from rural to urban areas will increase the obesity, adiposity and cardiovascular diseases compared to non-migrant children.

Of 659 children 121 (18%) had migrated to urban areas by 12y age. Socio-economic status (SES) of migrant children was higher (45% vs 29% Upper class) compared to non-migrants. On regression analysis, they had higher BMI (15.3 vs 14.8 kg/m2, $p=0.05$), MUAC (19.7 vs 18.5,$p=0.0001$), subscapular skinfolds (7.5 vs 6.8, $p=0.04$) and systolic blood pressure after adjusting for age and gender compared to non migrant children. They had lower hemoglobin levels (12.6 vs 12.8g/dL, $p=0.03$). Other metabolic and CVD risk factors were similar.

Migration into urban areas is associated with increase in obesity-adiposity, systolic BP and low hemoglobin concentration even as early as 12y of age. This was strongly associated with increase SES of the migrant children. Serial followup will reveal the effect on incident diseases.

PIII-402

Heavier Babies in the Northern US Predicts Higher Rate of C-Sections Due to Cephalopelvic Disproportions (CPD). Jagjit S. Teji[1], Ranjit K. Teji[2], Stephen Co[3]. *[1]Pediatrics, University of Chicago, IL, USA; [2]Pediatrics, Mercy Hospital and Medical Center, IL, USA; [3]Pediatrics, Loyola University, IL, USA.*

Previous reports have demonstrated a higher incidence of higher mean birthweight (bwt) in the northern states of US due to temperature (T) being lower. The purpose of this study is to determine whether the incidence of CPD is higher and consequently higher complications in the northern states in the US.

Linked Infant death and birth perinatal file from the NCHC for the year 2002 was used as data. The usual variables were employed for the analysis with respect to outcome variables CPD and c-section (CS) and state of birth and average temp annually in the respective states. Stats 10.0 was used for analysis.

There were over four million birth in the year 2002. Mean bwt of babies born in the northern states in the US with mean T less than 55°F was significantly higher OR 1.2, $p< 0.0005$. While controlling for the comfounding variables the Incidence of CPD and CS was significantly higher $p< 0.005$ for both in the northern states. Incidence was significantly higher in the Asian races for higher mean bwt of the newborn.

This is the first report of higher incidence of CPD and CS in the northern states. Implications of colder weather in the northern states may have increased adverse effects during perinatal period due to higher mean bwt of the newborns especially in the Asian races.

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PIII-403

Prenatal Stress (PS) Accelerates Maturation of the Autonomous Nervous System (ANS) and Induces Sympathetic Hyperactivity. Florian Rakers[1], Vilmar Frauendorf[3], Sven Rupprecht[1], Rene Schiffner[1], Harald Schubert[2], Sabine Bischoff[2], Matthias Schwab[1]. *[1]Dept.of Neurology, Friedrich Schiller University Jena, Germany; [2]Institute of Lab Animal Sciences, Friedrich Schiller University Jena, Germany; [3]Dept. of Hepatology and Gastroenterology, Charité Berlin, Germany.*

PS and glucocorticoid exposure program hyperactivity of the hypothalamic pituitary adrenal axis (HPAA). Effects on programming of the ANS are less clear. Our objective was to examine the direct effects of PS on maturation and programming of the ANS.

Seven pregnant ewes were isolated twice weekly for 3h between 30 and 100 dG (days gestation, term 150dG) resulting in a reproducible acute maternal cortisol increase. Eight pregnant ewes served as controls. After fetal instrumentation, electrocardiogram was monitored between 112 and 130 dG. Fetal heart rate variability (FHRV) was calculated in the time (SDNN, RMSSD) and frequency domain (LF, HF). Baroreceptor (BR) reflex response was derived at 112 and 130 dG from blood pressure (BP) responses to phenylephrine (PE) and sodium nitroprusside (SNP).

Fetal heart rate (FHR) did not change in controls between 112 and 130 dG (179±6 vs. 180±5 bpm, SEM) and decreased in PS fetuses (183±5 vs. 159±6 bpm, $p<0.05$, SEM). The SDNN/RMSSD ratio and the LF (vagal and sympathetic modulation) were higher in PS fetuses than in controls at 112 and 130 dG ($p<0.01$)suggesting higher total autonomous tone and sympathetic activity. The LF/HF ratio (sympathovagal balance) increased at 130dG ($p<0.05$) suggesting an increased sympathetic tone.

Baseline BP and FHR did not differ between the groups and gestational ages (GA). In response to PE, the BR response was similar in control and stressed fetuses at both GA. In response to SNP, the BR response was also similar but the maximal FHR increase was higher in the stressed fetuses at both GA ($p<0.05$). Three out of seven controls but none of the stressed fetus lacked a BR response to SNP.

PS at early and mid gestation induces early maturation of the ANS that is reflected by a higher autonomous tone at 112dG. The autonomous tone of the controls increased from 112 to 130 dG to this level. Regardless, the sympathetic tone of the PS fetuses was higher than in controls at both gestational ages suggesting programming of the stress reactivity of the ANS. Since the increased sympathetic cardiac control is not accompanied by a changed BR reflex sensitivity, prenatal stress does not seem to affect vagal cardiac control.

PIII-404

Higher Environmental Temperature during Pregnancy Causes Low Birthweight (LBW). Jagjit S. Teji[1], Alka Gupta[2], Kamal Eldeirawi[3]. *[1]Pediatrics, University of Chicago, IL, USA; [2]Medical School, University of Illinois, IL, USA; [3]College of Nursing, University of Chicago, IL, USA.*

Background: LBW is a major cause of infant morbidity and mortality in the world. Animal and human studies have shown that intrauterine growth retardation was inversely related to environmental temperature.

Objective: The purpose of this study is to assess the associations of higher temperature and lower latitude with lower birth weights in the United States infant born between 1995 and 2002.

Design/Methods: The data form CDC Linked Death and Infant Birth Data, from 1995 through 2002. Analyzed only term babies. Other data from MaxMind and National Oceanic and Atmospheric Administration (NOAA) website; The variables from the NCHS file used were maternal age, race, and Hispanic origin of the parents, birth weight, period of gestation, plurality, prenatal care usage, maternal education, live birth order, marital status, and maternal smoking and alcohol usage. Logistical regression was performed with LBW as the dependent variable with all the other dicotomized variables as confounding and where independent variables latitude and/or temperature with respect to race using STATA 9.0 SE.

Results: About 25 million records from over 32 million births were used for analysis. It was noted average annual temperature>55 F and lower latitude had a higher probability of LBW; Odds ratio (OR) 1.228029, $p<0.0005$; 95% CL-1.22-1.23). Greatest impact of LBW was for NHW and lowest for H, OR 1.22 vs 1.003. Other variables had no impact on the probability of LBW when inversely related to temperature and/or lower latitude of birth.

Conclusions: 1. LBW at birth is directly related to higher environmental temperature during pregnancy. 2.Being born LBW has direct association with adult onset diseases.

PIII-405

Prenatal Psychosocial Stress and the Child's Blood Pressure and Hypertension at Age Five. Aimée E. van Dijk[1,2], Manon van Eijsden[2], Karien Stronks[1], Reinoud J. Gemke[3], Tanja G. Vrijkotte[1]. *[1]Department of Public Health, Academic Medical Center, Amsterdam, Netherlands; [2]Department of Epidemiology, Documentation and Health Promotion, Public Health Service of Amsterdam, Netherlands; [3]Department of Pediatrics, VU University Medical Center, Amsterdam, Netherlands.*

Evidence from both animal and epidemiological studies suggests that prenatal stress has permanent effects on offspring structure and function, which may predispose to cardiovascular diseases. Our objective was to study maternal psychosocial stress as a potential prenatal factor associated with blood pressure in the child.

Pregnant women in the Amsterdam Born Children and Their Development (ABCD) study completed a questionnaire (around gestational week 14) including information on psychosocial stressors: depressive symptoms, pregnancy related anxiety, parenting daily hassles & job strain. Scores above the 80th percentile were considered high and summed up to a cumulative stress rank score (0-4). Systolic and diastolic blood pressure (BP) and Mean Arterial Pressure (MAP) were measured in the 5-year-old offspring. Hypertension was defined by using external gender and height-specific 95th percentiles. Only mothers with single births who were not using anti-hypertensive medication and who had children without reported heart problems were included (N=3,148).

After adjustment for relevant confounders, the accumulation of three or four psychosocial stressors prenatally (3%) was associated with 1.9 mmHg higher diastolic BP (95%CI 0.2;3.6) and 1.8 mmHg higher MAP in the offspring (95%CI 0.3;3.3) compared to those with no prenatally reported stressors (53%). This did not increase the risk for hypertension (OR 1.8; 95%CI 0.9;3.7). One (32%) or two (12%) reported psychosocial stressors was not associated with BP in the child. The associations were not different between boys and girls.

Accumulation of psychosocial stressors during pregnancy appears to be associated with higher diastolic blood pressure and mean arterial pressure in the child already at age five. Considering the putative role of high blood pressure as a causal factor in the development of CVD, prevention of maternal stress in the child's early stages of life may be valuable to improve cardiovascular health later in life.

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Prenatal Stress and the Child's Cardiac Autonomic Nervous System Balance at Age Five. Aimée E. van Dijk[1,2], Manon van Eijsden[2], Karien Stronks[1], Reinoud J. Gemke[3], Tanja G. Vrijkotte[1]. *[1]Department of Public Health, Academic Medical Center, Amsterdam, Netherlands; [2]Department of Epidemiology, Documentation and Health Promotion, Public Health Service (GGD) of Amsterdam, Netherlands; [3]Department of Pediatrics, VU University Medical Center, Amsterdam, Netherlands.*

Autonomic nervous system (ANS) disbalance is a potentially causal factor in the development of CVD. The ANS may be programmed during pregnancy due to maternal factors. Our aim is to study prenatal maternal psychosocial stress as a potential disruptor of cardiac ANS balance in the child.

Mothers from a prospective birth cohort (ABCD study) filled out a questionnaire around gestational week 16, including validated instruments for depressive symptoms, pregnancy related anxiety, parenting daily hassles and job strain. A cumulative stress score was calculated (possible scores 0-4, based on 80th percentiles). Indicators of cardiac ANS in the offspring at age five are: pre-ejection period (PEP), heart rate (HR), respiratory sinus arrhythmia (RSA) and cardiac autonomic balance (CAB), measured with electrocardiography and impedance cardiography in resting supine and sitting position. 3,035 mother-child pairs, only single births, were available for analysis.

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Accumulation of maternal stress was not associated with HR, PEP, RSA and CAB (p=0.15; p=0.27; p=0.29 and p=0.11 respectively). There was no significant effect-modification by smoking during pregnancy, sex of the fetus or prematurity. Birth size was only an effect-modifier in PEP (p=0.04), but patterns were unclear.

Accumulation of prenatal stress did not appear to be associated with the child's cardiac ANS in rest. Results were not supportive of the hypothesis that prenatal maternal psychosocial stress deregulates cardiac ANS balance in the offspring, at least in rest, and at age five.

perturb epigenetic patterns) might explain observed associations. Genetic variation, in the form of single nucleotide polymorphisms (SNPs), is robust to reverse causation as a disease state cannot alter genotype and can be used to infer the direction of causality.

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

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

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

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