Title: Sex- and Dose-Specific Transgenerational Effects of Bisphenol A on Metabolic Health

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Background: Ubiquitous exposure to an endocrine disruptor, Bisphenol A (BPA), is associated with health abnormalities in immediate and subsequent generation. However, transgenerational effects of BPA on metabolic health are unknown. We recently demonstrated that maternal (F0) BPA exposure has sex- and dose-specific effects on pancreatic islets of the first (F1) and second generation (F2) mice offspring. We extended our analysis to the third generation (F3) to determine the transgenerational effects of BPA on pancreatic islets.

Methods: Weekly body weights, glucose tolerance, and body composition by DEXA were assessed in F3 male and female offspring (10 µg/kg/day, LowerB; 10 mg/kg/day, UpperB; 7% corn oil diet, Control; n=10-12 litters/group). In n=4-8 littters/group, we determined: glucose stimulated and mitochondrial driven insulin secretion (islet perifusion ramps); ß-cell mass and proliferation (insulin-Ki67 immunofluorescence staining); cell death (caspase 3 activity fluorometric assay); presence of T-lymphocytes and macrophages (CD3 and F4/80 immunostaining); and cytokine/chemokine profile (Luminex assay). Each BPA group was compared with Control by Dunnett's multiple comparison test; $p<0.05$ considered significant.

Results: F3 LowerB and UpperB females had comparable body weight, glucose tolerance and insulin secretion as Controls. In contrast, F3 LowerB and UpperB males had moderately increased body weight and fat mass, while glucose tolerance and mitochondrial driven insulin secretion was similar to Controls. F3 LowerB, but not UpperB, males had increased glucose stimulated insulin secretion and ß-cell proliferation, which appeared compensatory to reduced ß-cell mass observed in LowerB group. Both BPA groups had increased CD3 and F4/80 staining in pancreas, but this was not associated with increased proinflammatory cytokine levels.

Conclusion: Maternal BPA exposure has sex- and dose-specific, but relatively mild, effects on metabolic health of F3 offspring. While some effects observed in F1 and F2 offspring persist in F3s, most effects disappear. Interestingly, F3 offspring appear to develop compensatory mechanisms to BPA exposure.

Glyphosate Exposure Levels in Pregnant Women: An Indiana Birth Cohort Study

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Background: Glyphosate (Roundup®), the most heavily used herbicide worldwide, is a known endocrine-disruptor that may have consequences for fetal development. Despite its widespread use in agricultural and residential landscapes, no direct measures of glyphosate exposure have been made in pregnancy. We conducted a prospective cross-sectional cohort study to measure glyphosate exposure in pregnant women to identify potential exposure pathways and to evaluate its association with pregnancy outcomes.

Methods: Urine and residential drinking water samples were collected from 71 women with singleton pregnancies at two private obstetrical practices in Indianapolis. Glyphosate measurements were performed using LC-MS/MS with a lower limit of quantification of 0.5 and 0.005 ppb in urine and water, respectively. Demographic and survey information relating to food and water consumption were obtained by questionnaire. Maternal risk factors and neonatal outcomes were abstracted from medical records. Correlation analyses were used to assess relationships between glyphosate levels and fetal growth and pregnancy outcomes.

Results: Maternal race was 94.2% Caucasian and 5.8% Asian. Glyphosate residues were detected in 93% of subjects (mean ± SD; 3.4 ± 1.2 ppb), but all drinking water samples were negative. Higher glyphosate levels were significantly associated with shorter gestational length ($r = -0.27$, $p=0.02$), borderline significant for lower birth weight percentile ($r = -0.20$, $p=0.09$), and nonsignificant for head circumference ($r = -0.08$, $p=0.50$). Higher glyphosate levels were found in women who lived in rural areas ($p=0.004$) and in those who consumed >24 oz of caffeinated beverages per day ($p=0.005$).

Conclusions: Glyphosate exposure was found in nearly all study participants and a significant association was found between urine glyphosate levels and shorter pregnancy length. The high prevalence of glyphosate exposure in pregnant women raises concern for harm to the developing fetus. Further research is warranted to determine the true risk to the mother, her fetus, and its future offspring.

ABSTRACT - US DOHaD Society annual meeting, Sept 25-27, 2017, Detroit, MI

Diabetogenic Risk in Young Adults Exposed to Perfluoroalkyl Substances over the Lifecourse.

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Background: Animal models show hepatotoxic and diabetogenic effects caused by developmental exposures to perfluoroalkyl substances (PFASs). Population studies with prospective exposure assessments are needed to test this hypothesis.

Aim: We evaluated the prospective associations of PFAS exposure in utero, childhood, puberty, and early adulthood with diabetogenic markers in young adults.

Methods: We analyzed 636 Faroese individuals followed prospectively from birth (in 1986/7) to age 28 years (62% of initial cohort). PFAS concentrations were measured in cord whole blood and participant's serum at ages 7, 14 and 22 years. Using a 2-hour (75g) oral glucose tolerance test administered at age 28, we calculated clinical indices of insulin sensitivity (i.e. homeostatic model assessment of insulin resistance [HOMAIR], Matsuda insulin sensitivity index [ISI]), and insulin secretion (i.e. corrected insulin response [CIR], insulinogenic index [IGI] and insulin- and glucose- areas under the curve [AUC]). Association coefficients from linear regression models adjusted for significant confounders are expressed as % change in the geometric mean (GM) of the clinical indices per doubling of PFAS concentrations.

Results: PFAS concentrations at all life periods were higher for PFOS followed by PFOA, PFHxS, PFNA and PFDA. Concentrations of PFOS, PFOA and PFHxS decreased over time, while concentrations of PFNA and PFDA increased. Cord blood PFOS concentrations were associated with lower ISI (%GM change per PFOS doubling [95%CI]: -5% [-11, 2]) and higher HOMAIR (4% [-4,12]), CIR (14%; [4, 25]), IGI (17% [6, 30] and insulinAUC (8% [1, 15]), with some associations being stronger in women compared to men (P- sex interaction<0.10 for CIR and IGI). Associations of same direction and smaller magnitude were seen for PFOS concentrations measured at later ages. Less conclusive association patterns were found for other PFASs.

Conclusion: Lifecourse exposure to PFASs and especially exposure *in utero* is associated with clinical markers of insulin resistance and impaired pancreatic beta-cell function in healthy young adults.

Preconception as a Sensitive Window of Exposure: Urinary Concentrations of Organophosphate Flame Retardant and Fertility Outcomes among Couples Undergoing in Vitro Fertilization

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Use of organophosphate flame retardants (PFRs) has increased over the past decade with the phase out of some brominated flame retardants. We recently reported associations of some urinary PFR metabolites with decreased proportions of fertilization, implantation, clinical pregnancy and live birth among women recruited from an academic fertility clinic. In this analysis, we report on urinary concentrations for the male partners, examine predictors of these concentrations, and examine associations between urinary concentrations of PFR metabolites and outcomes of *in vitro* fertilization in their partner. Our analysis included 209 couples enrolled in the Environment and Reproductive Health (EARTH) prospective cohort study (2005-2015). We measured five urinary PFR metabolites using negative electrospray ionization liquid chromatography tandem mass spectrometry. We used multivariable generalized linear mixed models to evaluate the association of demographic characteristics with the PFR metabolites and PFR metabolites with IVF outcomes, accounting for multiple IVF cycles per couple. Detection frequencies were high (>75%) for BDCIPP, DPHP and ip-PPP but low (<15%) for tb-PPP and BCIPP. Some PFR urinary metabolites were associated with race, body mass index, year of treatment cycle, and season. An 8% decline was observed for the highest compared to lowest quartile of urinary BDCIPP in adjusted means for cycles resulting in successful fertilization (adjusted absolute difference=0.06 (0.01, 0.12), p-trend=0.06), after adjusting for maternal exposure. We conclude that male partner urinary concentrations of BDCIPP may be associated with fertilization whereas *maternal* urinary DPHP, ip-PPP and ΣPFR account for negative associations previously observed for proportions of successful fertilization, implantation, clinical pregnancy and live birth. These results highlight potential reproductive effects of exposure to PFRs at levels currently common among the general population.

Title: Placental expression of imprinted genes associated with cadmium exposure, primarily among male placental tissue.

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Background: Imprinted genes have parent-of-origin specific mono-allelic expression, are highly expressed in placental tissues, and play critical roles in fetal growth and development. Thus, disruptions to the regulation of imprinted genes during pregnancy may have negative consequences on fetal development. We tested whether imprinted gene expression was associated with placental concentrations of the developmentally toxic metal cadmium (Cd) in two population-based studies.

Methods: We examined the relationships between the expression levels of 69 putative imprinted genes (expressed in placental tissues) in relation to concentrations of log-transformed Cd in placental tissue using linear regression, while adjusting for potential confounders, across two independent samples: the New Hampshire Birth Cohort Study (NHBCS, n=326) and the Rhode Island Child Health Study (RICHS, n=157). We also explored whether Cd-associated variations in expression were sex-specific. Results across the two cohorts were aggregated via inverse variance weighted fixed effects meta-analysis.

Results: Cadmium concentrations in placental tissues were associated with four imprinted genes after Bonferroni-adjustment (p-values < 0.00072). Higher Cd concentrations were associated with lower expression of *H19* (β_1 = -0.45, p-value = 0.0000077) and *IGF2* (β_1 = -0.47, p-value = 0.00019), and with higher expression of *DLX5* (β_1 = 0.40, p-value = 0.000029) and *GAA* (β_1 = 0.34, p-value = 0.00025). All four genes demonstrated strong effects in male placental tissues, but only *DLX5* was significantly associated with Cd in female placental tissues.

Conclusion: Of note, higher placental cadmium was significantly associated with lower expression of *H19* and *Igf2*, particularly among male placentae. These genes are regulated by the same imprinting control region and dysregulation of *H19* and *IGF2* can lead to growth restriction or developmental disorders which can have long-term impacts on health. These findings provide evidence of a possible mechanism through which Cd may impart some developmentally toxic effects on human pregnancies.

Aberrant estrogen metabolism in preeclampsia may cause dysregulation of angiogenesis-mediated uterine blood flow via a novel uterine endothelial sympathomimetic system.

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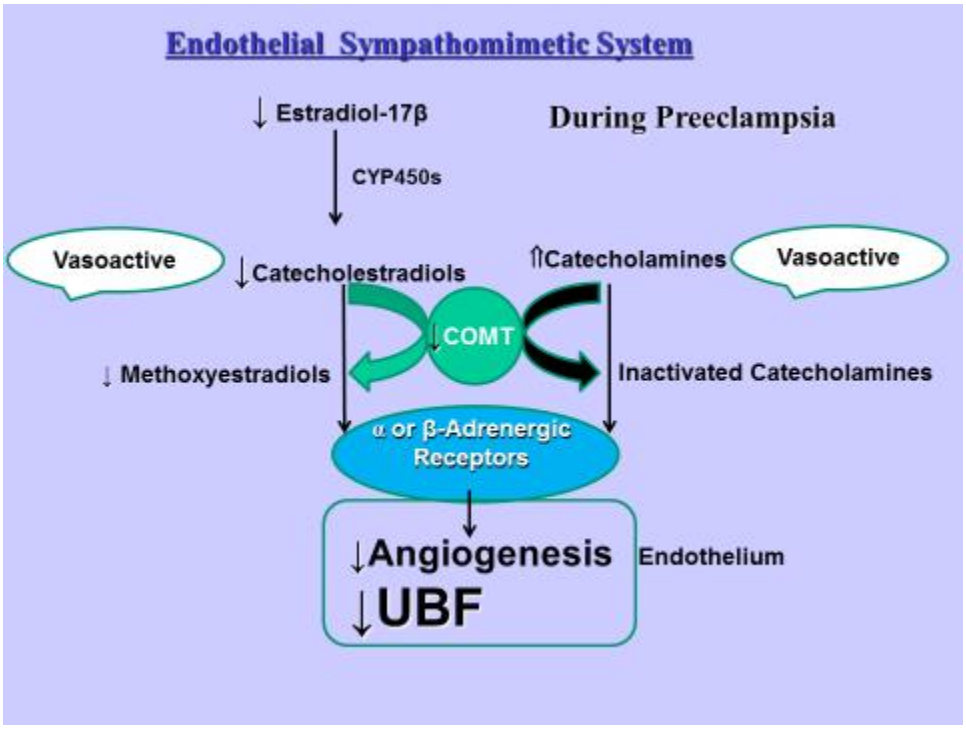
Background: Uterine angiogenesis mediates gestational UBF rises and fetal nutrient delivery. Estradiol-17β and its cytoP450s and catechol-*O*-methyltransferase(COMT) metabolites; 2-Hydroxyestradiol(OHE_2), 4-OHE_2, 2-Methoxestradiol(ME_2), and 4-ME_2 are elevated during pregnancy, but reduced in preeclamptic (PE) pregnancies. COMT knockout mice develop PE-like symptoms. **Methods:** CytoP450s, COMT, Estrogen Receptors (ER) and Adrenergic Receptors (AR) levels were evaluated in pregnant ovine Uterine Artery Endothelial Cells (P-UAECs). P-UAEC proliferation was measured with 0.1-100 nmol/L of E_2β,2-OHE_2,4-OHE_2, 2-ME_2,4-ME_2, norepinephrine, and epinephrine. ERα or ERβ versus ARα or ARβ on mitogenic responses were evaluated. Because catecholestradiols and catecholamines exhibit structural similarities and affinity for ARs, we **hypothesize** existences a novel uterine endothelial sympathomimetic system and investigated if endothelial ARα/ ARβ mediate proliferation of P-UAECs.

Results: LCMS on plasma from normotensive pregnant (normP;n= 8) and preeclamptic (PE;n=16) women revealed that PE had lower estrone, estradiol-17β, estriol, 2&4 hydroxyl catecholestrogens, and the COMT metabolites 2&4 methoxyestrogens. Western analyses on P-UAECs revealed cytoP450s,COMT, ERα,ERβ, $ARα_2$,$ARβ_2$ and $ARβ_3$, but not $ARα_1$ and $ARβ_1$. E_2β,2-OHE_2,4-OHE_2, and 4-ME_2, but not 2-ME_2 concentration-dependently stimulated* proliferation in P-UAECs. Proliferative responses to E_2β, but not its metabolites, were solely mediated by ERβ (not ERα). Propranolol (ARβ blocker), but not phentolamine (ARα blocker)

suppressed* norepinephrine-,epinephrine-, and Catecholestradiol-induced P-UAEC proliferation. AR β_2 and ARβ_3 antagonists (ICI 118,551 and SR 59230A) abrogated* 2-OHE_2 and 4-OHE_2 mitogenesis; 4-ME_2 proliferation was neither ER nor AR mediated.

Conclusions: Aberrant synthesis/metabolism of estrogens and estrogen metabolites in PE provide clinical insight for steroid links to reduce UBF in PE gestations. We report a novel uterine endothelial sympathomimetic system (Figure) demonstrating functional roles for $E_2\beta$, and its cytoP450- and COMT-derived metabolites in angiogenesis regulation that may be dysfunctional in PE. Thus important convergences of endothelial ER and AR systems regulate angiogenesis, thus providing distinct evolutionary advantages for positively modulating uterine perfusion during stressful pregnancies. *P<0.01



Transcriptomic analysis reveals novel mechanisms mediating islet dysfunction in the intrauterine growth restricted (IUGR) rat

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ABSTRACT

Intrauterine growth restriction (IUGR) increases the risk of developing Type 2 Diabetes in adulthood. Previous studies employing bilateral uterine artery ligation in our rat model of IUGR have revealed age-associated decline in glucose homeostasis and islet function. To elucidate novel mechanisms contributing to IUGR pathogenesis, the islet transcriptome was sequenced at 2 weeks of age, when *in vivo* glucose tolerance is mildly impaired, and at 10 weeks of age, when rats are hyperglycemic and have reduced β-cell mass. Islets from IUGR rats and controls were isolated with collagenase digestion followed by histopaque gradient centrifugation. RNA-Seq Libraries were single-end sequenced to 100 bp, aligned using Tophat and analyzed using EdgeR. Differential gene expression was defined as having cpm > 4, fold change > 1.5, and false discovery rate < 0.05. RNA sequencing and functional annotation of differentially expressed genes using

Ingenuity Pathway Analysis reveal novel temporal changes in IUGR islets. Gene expression involving amino acid metabolism was reduced primarily at 2 weeks of age but ion channel expression, specifically those involved in cell-volume regulation, was more disrupted in adult IUGR islets. Additionally, we observed alterations in the microenvironment of IUGR islets with ECM genes being increased at 2 weeks of age and decreased at 10 weeks. Specifically, hyaluronan synthase 2 expression and hyaluronan staining were increased in IUGR islets at 2 weeks of age. Mesenchymal stromal cell-derived factors that have been shown to preserve islet allograft function, such as *Anxa1*, *Cxcl12,* and others, were also increased at 2 weeks and decreased in adult islets. Finally, comparisons of differentially expressed genes to those of type 2 diabetic human islets support a role for these pathways in human diabetic patients. Taken together, these data point to new mechanisms in the etiology and pathogenesis of IUGR-mediated islet dysfunction in Type 2 Diabetes.

The association between placental inflammation and BMI at age 7

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Background: A growing body of evidence demonstrates an association between the intrauterine environment and childhood overweight and obesity; however, the mechanism underlying these associations remains poorly defined. Given the critical role of the placenta in determining the intrauterine environment, we posit that prenatal exposure to placental inflammation may predispose offspring to the development of overweight or obesity later in life.

Methods: Data were from the US Collaborative Perinatal Project, a prospective cohort study that enrolled women prenatally at 12 sites (1959-1965) and followed the children until age 7. The association between placental inflammation and body mass index (BMI) at age 7 was examined in 30,444 singleton births using generalized estimating equations in order to account for variation across multiple sites. Only singleton births between 20 and 42 weeks gestation were included in the analysis. Gross and histological assessment of the placenta were done by trained pathologists using standardized protocols. Acute and chronic placental inflammation were dichotomized as present or absent at each anatomical site assessed. Adjusted models controlled for race, infant birthweight, and infant sex.

Results: Acute inflammation in Wharton's jelly (B = 0.24; 95% confidence interval (CI) = 0.13, 0.35), the umbilical vein (B = 0.13; 95%CI = 0.06, 0.21), and umbilical artery (B = 0.25; 95%CI = 0.13, 0.36) were all associated with increased BMI at age 7 even after adjusting for potential confounders. Acute inflammation in the vessels on the chorionic plate was also associated with BMI at age 7 after adjusting for confounders, but chronic inflammation, regardless of location in the placenta was not associated with an increased BMI at age 7.

Conclusion: These results suggest that prenatal exposure to acute placental inflammation may predispose offspring to overweight and obesity beginning in childhood.

Associations between Maternal Early Pregnancy Body Mass Index (BMI) and Placental DNA Methylation at Term

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Background: The placenta serves as the definitive maternal-fetal interface and mediates exchange of nutrients, gases, and waste between mother and the developing fetus. The placenta integrates signals from both mother and baby, coordinating maternal nutrient supply with fetal demand and development. In epidemiological studies, maternal obesity and obesogenic diets are associated with fetal overgrowth and greater risk of obesity and metabolic dysfunction in later-life. Animal and human studies have shown maternal obesity-associated alterations in placental gene expression and implicated altered placental nutrient transport in excessive fetal growth and developmental programming.

Hypothesis: We hypothesized that placental epigenetic patterns are associated with maternal early pregnancy BMI or maternal diet composition. Maternal BMI was assessed at <10 weeks of gestation and maternal macronutrient diet composition was assessed by 3-day food records every 6 weeks during pregnancy.

Methods: Using Reduced Representation Bisulfite Sequencing (RRBS), we digitally assessed genome scale DNA methylation of approximately 300,000 CpGs in 150 placenta (N=72 normal weight mothers, N=78 overweight/obese mothers).

Results: In multivariable linear regression models adjusted for multiple testing and covariates (maternal age at delivery, infant sex, and delivery mode), maternal early pregnancy BMI, but not maternal percent of calories from fat, protein, and carbohydrates was significantly associated with placental DNA methylation of specific CpGs. Of the 54 CpGs significantly associated with maternal early pregnancy BMI ($q<0.02$), gene ontology analysis of the proximal genes associated with these differentially-methylated CpGs showed biological processes related to metabolism and development to be affected, and molecular functions such as protein, nucleotide, chromatin, and lipid binding, and transporter activity to be differentially methylated.

Conclusions: These data suggest that maternal obesity, as assessed by early pregnancy BMI, has a greater effect on placental DNA methylation patterns than maternal diet composition. This work was supported by USDA ARIS Project 6026-51000-010-05S.

Maternal depression and anxiety on placental imprinted gene expression

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Background:
Maternal depression - which affects up to 30-40% of pregnant women - and anxiety, have been associated with impaired fetal growth and development, preterm birth, and poorer neurodevelopmental outcomes of the offspring. The placenta, which regulates the infant nutrient and hormonal environment, may modulate much of the effects of maternal stress on the fetus. In the placenta, imprinted genes play a central role in regulating nutrient transport, infant growth and neurodevelopment.

Hypothesis:
We hypothesized that women with depression/anxiety would have differences in imprinted gene expression and that this variation can contribute to developmental outcomes that have been previously linked to prenatal stressors.

Methods:
Imprinted gene expression was profiled in placental tissue from the Rhode Island Children's Health Study using the NanoString® (Seattle, WA) platform and expression patterns were compared using linear models between those who reported a history of both depression and anxiety/OCD/panic during pregnancy (n=54), in comparison to women with no reports of psychiatric history (n=458).

Results:
Twenty-one genes were identified as being significantly differentially expressed between the depression/anxiety group and the controls using an FDR correction. Most these genes (76%) had lower expression in placentae from women with depression/anxiety than controls, with a range of log fold changes between -0.11 and -0.79. These differences remained when the case group was restricted to women who were taking psychiatric medication during pregnancy. Three of the genes with the largest decreases in expression—*PLAGL1*, *NDN*, and *DLK1*—were also significantly decreased in the depression group compared with controls. Among those with depression, we found no difference in expression between those who were and were not taking a medication.

Conclusions:
Overall, stress during pregnancy may be related to changes in placental function through regulation of imprinted genes. Further analyses suggest the relationship was associated with the stressor and not pharmaceutical treatment.

Associations of cord blood metabolites with newborn anthropometry and cord blood hormones in Project Viva

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Background: Metabolomics is a powerful tool to characterize biomarkers and elucidate pathophysiological processes. Little is known of these relationships during gestation and infancy, which are sensitive periods for metabolic disease risk. We aimed to identify cord blood metabolites associated with birth size (birthweight for sex and gestational age z-score [BW/GA]), and to examine their associations with cord blood hormones implicated in growth and metabolism: leptin, adiponectin, insulin-like growth factor [IGF]-1 and IGF-2.

Hypothesis: Cord blood metabolites on lipid and amino acid pathways will be associated with larger birth size, higher leptin, lower adiponectin, and higher IGF-1 and IGF-2.

Methods: Among 126 participants in Project Viva, a U.S. pre-birth cohort, we used untargeted mass spectrometry to quantify metabolites in cord plasma. After excluding 103 xenobiotic metabolites, we used principal components analysis to consolidate the remaining 606 endogenous compounds into principal components (“factors”). Next, we identified factors associated with BW/GA after Bonferroni’s correction. Finally, we examined relations of the BW/GA-associated factors with cord blood insulin, leptin, adiponectin, IGF-1 and IGF-2 using linear regression models that accounted for

mother’s race, delivery mode, and infant sex and gestational age at birth. Models for IGF-1 or IGF-2, were further adjusted for IGF binding protein-(IGFBP)-3.

Results: Mean BW/GA was 0.27±0.98 units. Approximately half of the infants were male (52.4%) and white (57.1%). Of the 6 factors retained from PCA, one was associated with higher BW/GA (0.28 [95% CI: 0.12, 0.43]). This factor comprised metabolites involved in amino acid (spermidine, aspartate) and RNA (inosine 5-monophosphate, adenosine 5-monophosphate, cytidine 5-monophosphate) pathways. In multivariable analysis, this factor corresponded with higher cord blood leptin (1.49 [0.28, 2.70] ng/mL) and IGF-1 (3.34 [0.20, 6.49] ng/mL).

Conclusions: Amino acid metabolites and compounds on RNA pathways in cord blood are associated with larger size at birth, as well as higher leptin and IGF-1.

K Varberg Abstract

Fetal exposure to maternal gestational diabetes mellitus (GDM) predisposes children to future health complications including hypertension and cardiovascular disease. A key mechanism by which these complications likely occur is through the functional impairment of vascular progenitor cells, including endothelial colony forming cells (ECFCs). Previously, we showed that fetal ECFCs exposed to GDM have decreased vasculogenic potential and altered gene expression. In this study, we evaluate whether Transgelin 1 (TAGLN), which is increased in GDM-exposed ECFCs, contributes to vasculogenic dysfunction. TAGLN is an actin-binding protein involved in regulation of cytoskeletal rearrangement. We hypothesized that increased TAGLN expression in GDM-exposed fetal ECFCs decreases network formation through altered migration due to impaired cytoskeletal rearrangement. To determine if TAGLN is required and/or sufficient to impair ECFC network formation, TAGLN was reduced and overexpressed in ECFCs from GDM and uncomplicated pregnancies, respectively. Decreasing TAGLN expression in GDM-exposed ECFCs increased network formation ($n=8$, $p=0.019$) and cell migration ($n=10$, $p=0.028$) in addition to altering network formation kinetics ($n=5$, $p<0.05$). In contrast, overexpressing TAGLN in ECFCs from uncomplicated pregnancies decreased network formation ($n=10$, $p=0.047$) and cell migration ($n=11$, $p=0.015$), altered network formation kinetics ($n=6$, $p<0.05$), and impaired ECFC alignment to laminar flow in shear stress assays ($n=3$, $p=0.0037$). Both ECFCs from GDM pregnancies and ECFCs overexpressing TAGLN have increased phosphorylation of myosin light chain. However, reducing myosin light chain phosphorylation via Rho kinase inhibition increased migration of GDM-exposed ECFCs ($n=6$, $p=0.01$) and ECFCs overexpressing TAGLN ($n=5$, $p=0.019$). Overall, these data suggest that increased TAGLN expression in GDM-exposed ECFCs is detrimental, as it reduces ECFC migration, cell alignment, and ultimately, network formation. Identifying the molecular mechanisms underlying ECFC dysfunction following intrauterine exposure to GDM, like TAGLN overexpression, will provide a greater understanding of subsequent development of vascular complications later in life, and hopefully improve diagnosis and treatment.

Title: Programmed Adipocytes Contribute to Obesity in Low Birth Weight Offspring: Interaction with Ambient Temperature and Fat Depots

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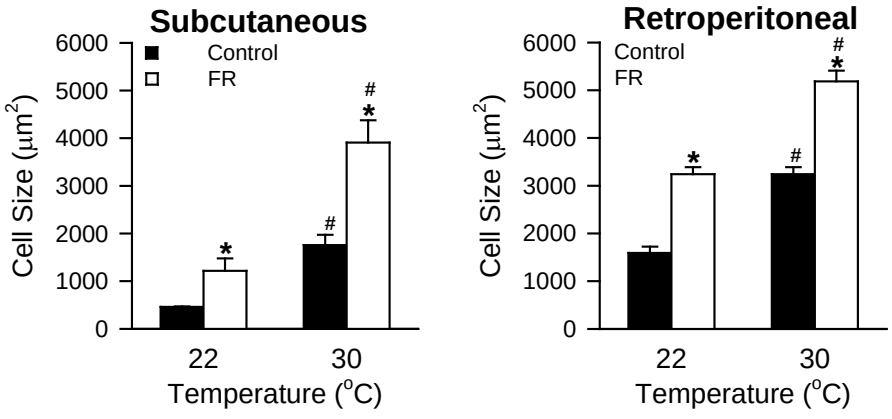
Background: Low birth weight newborns (LBW) have increased risk of adult obesity, in part due to enhanced adipogenesis as a result of hyperplasia and/or hypertrophy of adipocytes. Ambient temperature (conventional 22°C; thermoneutral 30°C) is known to impact adipogenesis in both subcutaneous and visceral retroperitoneal fat. We hypothesized that adult LBW offspring will have increased adipocyte cell size and/or number, and that housing at thermoneutrality will accentuate the adiposity difference between LBW and Control offspring.
Study Design: Pregnant mice were housed at either standard 22°C or thermoneutral 30°C room temperature. At gestational age e10, mice were fed either an *ad libitum* diet (Control) or were 30% food-restricted to produce LBW newborns. Following delivery, all mice were fed *ad libitum* diet. At 12 months of age, male offspring body weights and body fat were obtained, and subcutaneous and retroperitoneal adipose tissue was collected for determination of adipocyte cell size. Differences between groups were analyzed using ANOVA.
Results: LBW male newborns were significantly growth restricted at birth (22°C: 1.80±0.04 vs 2.08±0.06g; 30°C: 1.70±0.05 vs 1.87± 0.02g). However, adult LBW were markedly heavier than controls (22°C: 30.9±0.5 vs 27.8±0.4g; 30°C: 32.2±0.3 vs 29.9± 0.5g) and had significantly greater percentage body fat (22°C: 13.6±1.0 vs 10.4±1.1%; 30°C: 19.4±0.8 vs 16.5± 1.0%). LBW males had significantly increased adipocyte cell size as compared to controls, with this effect accentuated at 30°C and in retroperitoneal fat (Figure; * LBW vs Control; # 30°C vs 22°C).
Conclusion: In LBW offspring, hypertrophic adipocytes likely contribute to increased fat storage and obesity. At thermoneutrality, despite birth weight being lower, the adult body weight and fat was increased. Adipocyte cell size was larger in retroperitoneal versus subcutaneous fat, with effects accentuated by thermoneutral housing. Thus, thermogenesis-mediated effects contribute to reduced adiposity and cell size at lower temperature.



Maternal high fat diet exposure during pregnancy and lactation alters trabecular bone structure in rat offspring

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The perinatal period appears to be critical for the programming of skeletal architecture and homeostasis, suggesting that maternal diet during this period may have profound and lasting influences on offspring bone structure and strength. We used a rat model of maternal high fat diet exposure during pregnancy and lactation to investigate bone outcomes in offspring. Dams were placed on either a high-fat diet (HF, n=6, 60% kcal fat, Research Diets D12492) or a sucrose-matched control diet (CH, n=6, 10% kcal fat, Research Diets D12450J), with all offspring weaned onto the CH diet at postnatal day (PND) 21. At PND1 and at 4 months of age (adult), 1-2 male and female offspring per litter were sacrificed and blood plasma, L5 vertebra, and left and right femur were collected for assessment of bone turnover markers and micro-CT analysis of bone microstructure. At PND1, male HF offspring exhibited an increased trabecular bone volume fraction ($p=0.01$) in the distal femur compared CH controls that was secondary to increases in trabecular number ($p=0.01$) and thickness ($p=0.02$). However, adult male HF offspring exhibited a dramatically reduced trabecular bone volume fraction at the same skeletal site ($p\leq0.001$), with decreased trabecular number ($p\leq0.001$) and thickness ($p=0.03$) compared to CH controls. Female HF offspring showed an increase in bone volume fraction (BV/TV% $p=0.02$) only at PND1 that was not different in adult females. These results suggest that maternal HF diet may have persistent consequences on skeletal bone development, regulation, and architecture in offspring, and that these consequences may be both sex- and developmental stage-dependent.

Male and female HF offspring display marked sex differences in response to cocaine

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Male and female high-fat (HF) diet offspring have a greater preference ratio for HF diet, a blunted locomotor response to amphetamine (2mg/kg, i.p.), and do not develop a conditioned place preference for food reward compared to chow offspring. Collectively, these data suggest that early HF exposure may result in aberrant responding to rewarding stimuli. Here, we assessed their behavioral response to cocaine using a behavioral sensitization paradigm. Briefly, male and female offspring of Sprague-Dawley rat dams fed either chow (CH; D12450J, Research Diets) or high fat (HF; 60%, D12492, Research Diets) during gestation and lactation were weaned onto CH at P21 and tested at 8 wks of age. Females were in Diestrus 1 on day 1 of behavioral sensitization and challenge. Rats (n=8/diet/sex) were acclimated to a locomotor activity monitor for 30min, injected with saline, 30min later given cocaine (10mg/kg, i.p.) and monitored for an additional 90min daily for 8d, followed by an 8d withdrawal period. Next, rats were challenged with cocaine following the procedures as described above, then injected with cocaine (5mg/kg, i.p.) monitored for 60min, followed by a higher dose of cocaine (10mg/kg, i.p.) for 90min. Only male HF offspring sensitize to repeated cocaine exposure (D1 vs D8, p=0.01), but display similar locomotor response to cocaine challenge after an 8d withdrawal period compared to CH offspring. Male CH and HF offspring do not differ in their peak response to cocaine across all testing days. Neither CH nor HF female offspring sensitize to cocaine; however, HF female offspring are hyporesponsive on day 1 (initial 50min activity, p=0.04) but do not differ in their response to varying doses of cocaine during a challenge. Taken with our previous findings, early HF diet exposure may result in differential programming of responses to different rewarding stimuli that may be sex-specific.

Background: Maternal endocrine disorders are prevalent co-morbidities during pregnancy that impact maternal health, fetal development, and childhood health outcomes. Limited data exists on changes in breast milk composition associated with gestational endocrine disorders. The early life influences from altered breast milk composition are hypothesized to impact developmental programming during the critical lactation period.

Hypothesis: Based on existing data from animal models, we hypothesize that impaired maternal insulin signaling in endocrine disorders will lead to changes in breast milk fat content and fatty acid profile. These changes may then impact infant growth outcomes.

Methods: We are enrolling a large prospective cohort study of mothers with gestational diabetes mellitus (GDM), type 1 DM, type 2 DM, obesity, and polycystic ovary syndrome (PCOS) in comparison to healthy mothers. The University of Michigan Institutional Review Board approved this study. On human breast milk samples, we performed total fat analysis by creamatocrit and fatty acid analysis by mass spectrometry. We evaluated milk hormone content by ELISA and evaluated microRNA biomarkers using OpenArray with linear modeling. We analyzed results by one-way ANOVA with post hoc multiple comparisons testing. We will correlate infant growth trajectory over 2 years with milk composition.

Results: We have enrolled 53 mother-baby pairs. We have found that breast milk total fat content and insulin levels are elevated in obese compared to healthy mothers. Breast milk fatty acid profiles revealed an increase in dihomo-γ-linolenic acid in obese mothers. We have also identified breast milk miRNAs trending towards significance as biomarkers of maternal endocrine disorder states. Review of infant growth from birth to 2 months revealed elevated body mass index in infants of mothers with gestational diabetes.

Conclusions: Preliminary data has shown alterations in breast milk fat levels and fatty acid content in maternal endocrine disorder states and variations in infant growth patterns.

Calculation of costs and the Burden of Disease (BoD) is useful in developing resource allocation and prioritization strategies in medicine and public health. While useful, the Disability-Adjusted Life Year (DALY) metric disregards subclinical dysfunctions; adheres to stringent causal criteria; and is hampered by gaps in exposure data, especially from industrializing countries. For these reasons, a recently calculated environmental BoD of 5.18% of the total DALYs is likely too low.

We combined and extended cost calculations for exposures to environmental chemicals, with a focus on neurotoxicants, where sufficient data are available to determine dose-dependent adverse effects on a population basis. As a complement to these health economic estimations, we used attributable risk valuations to assess the overall environmental BoD. For comparison with DALY estimates, we used a standard value of $50,000 for each DALY. However, DALY calculations are available on few environmental chemicals, and are mostly based on mortality and on impact and duration of clinical morbidity, while less serious though chronic conditions are usually ignored. Economic estimates based on available exposure information and dose-response data for specific neurotoxicants show a total cost that greatly exceeds the DALY estimates.

Our calculations suggest that environmental chemical exposures contribute very substantially to the Global Burden of Disease (GBD) and that current DALY calculations of the GBD severely underestimate the benefits that may be achieved by better control of environmental risk factors, particularly the neurotoxicants that adversely affect early brain development. By including toxicological and epidemiological information and data on exposure distributions, more appropriate results can be obtained from utilizing health economic analyses of the adverse effects associated with environmental chemicals that are toxic to brain development.

Title: Coordinated roles of iron-dependent PHD and JARID in early-life iron deficiency-induced adult neural gene dysregulation

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Abstract:
Early-life iron deficiency (ID) affects approximately 20-30% of all pregnant women and their offspring worldwide and causes long-term impairments in cognition and socio-emotional behaviors in adulthood despite iron therapy after the diagnosis of ID in infancy. These persistent abnormalities constitute a significant cost to society in terms of loss of education, job potential, and mental health. The underlying pathobiology for the persistent behavioral abnormalities has been ascribed solely to abnormal neural development and function (e.g., metabolism, dendritogenesis, and synaptogenesis) during critical periods in early life that are carried forward into adulthood. However, major gaps in knowledge remain, one of which pertains to the iron-dependent cellular mechanisms, by which early-life ID induces the dysregulation of genes critical for neuronal function in adulthood. Such knowledge is critical to advance the field in terms of therapeutic strategies to prevent the long-term negative effects of early-life ID on the developing brain. We investigate how cellular pathways driven by iron-containing dioxygenases alter long-term neuronal expression of major synaptic differentiation and plasticity genes, including brain-derived neurotrophic factor (BDNF). We leverage the necessity of iron for the catalytic activity of two well-known families of dioxygenases, prolyl hydroxylases (PHDs) and JmjC ARID-domain containing histone demethylases (JARIDs), to analyze the functional effect of early-life ID on these proteins in neural tissues. We assess whether PHDs and JARIDs are key iron-containing factors directly responsible for the changes in chromatin structures, resulting in the persistent dysregulation of genes critical for learning and memory.

Developmental exposure to potential neurotoxicants and children's cognitive function at 7 years

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Background: Exposure to mercury has been associated with adverse effects on child neurodevelopment. However, the majority of studies did not simultaneously adjust for the multiple potential environmental neurotoxicants correlated with mercury concentrations. We aimed to assess the association between developmental exposure to multiple pollutants and children's performance on the Boston Naming Test (BNT) at 7 years of age using a novel statistical approach.

Methods: Concentrations of mercury (Hg), organochlorine compounds (OCs) including polychlorinated biphenyls (PCBs) and pesticides, five perfluoroalkyl substances (PFASs), and total mercury (Hg) were measured in maternal and children's blood at 5 years (n=338). At 7 years, children underwent a battery of neuropsychological tests. We focused on the BNT because it was shown to be the most sensitive to prenatal mercury exposure. We used the G-formula, a maximum likelihood-based substitution estimator, combined with an ensemble learning technique (i.e. SuperLearner) to infer effect estimates for each pollutant while mutually adjusting for other environmental exposures at both ages, and relevant confounders.

Results: Prenatal Hg, perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) concentrations were associated with lower scores on the BNT. A one standard deviation increase in Hg, PFNA, and PFDA concentrations was associated with decreases in the BNT scores by 0.18 (95% Confidence Interval (CI): -0.28, -0.12), 0.08 (95% CI: -0.13, -0.04), and 0.25 points (95% CI: -0.27, -0.22). No significant association was found for other exposures prenatally or at 5 years. We did not find evidence of interactions between environmental exposures, nor effect modification by relevant characteristics such as sex or maternal education. Finally, all the relationships exhibited a linear shape.

Conclusions: Using a novel statistical approach combining ensemble learning techniques and causal inference, we confirmed previously reported associations between prenatal mercury exposure and lower cognitive function. Additional attention should be directed towards the potential neurodevelopmental effects of emerging PFASs.

Fetal Origins of Child Sleep Problems: The Role of Maternal Psychological Stress

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Background. Child sleep disorders are increasingly prevalent in the USA and recent data shows that poor sleep in early childhood is strongly associated with increased emotional difficulties. Identifying early predictors of sleep problems, starting *in utero*, is of critical importance for early prevention. Here, we investigated the fetal origins of sleep problems in 4-5 year-olds in an at-risk pilot sample from Detroit, MI (N=51; 30 boys). **Hypothesis.** We expected that children prenatally exposed to maternal psychological stress experience increased sleep disturbances and that these disturbances are, in turn, associated with decreased emotion regulation skills. **Method**. During pregnancy, mothers completed questionnaires about stress, including daily stress, worry, anxiety, and depression. These scales showed very high loadings and good fit to a one-factor model (χ^2=19.11, df=9,p=.02; CFI=.97; TLI=.96; RMSEA=.08). At age 4-5 years, mothers reported on child sleep issues using the Child Behavioral Checklist (CBCL; Achenbach & Rescorla, 2000) and emotion regulation using the Emotion Regulation Rating (ERR; Carlson & Wang, 2007) scale. **Results**. Maternal stress, represented as a single latent factor, was related to child sleep problems. Specifically, higher maternal prenatal stress was associated with increased sleep issues (r=.503, p<0.001). The effect remained significant after controlling for birth outcomes, child sex, maternal age, and postnatal maternal anxiety symptoms (t=3.649, p=0.001). As expected, both higher prenatal stress and increased child sleep problems were related to less emotion regulation skills (r=-.292, p=0.040; r=-.381, p=0.006, respectively). However, bootstrap mediation analysis with child sleep as a mediator yielded no significant results (95% CI [-1.620, .304]), probably due to low power. **Conclusion.** Our preliminary data indicates that sleep disturbances in early childhood may have a fetal origin and that they are likely to contribute to child emotion regulation problems. Prenatal factors should therefore be considered in future examination of early predictors of child sleep issues.

Keywords: prenatal; maternal stress; child sleep

Funding: MH110793 (MET), R21ES026022 (MET), R01HD075806 (MET), NARSAD (MET)

Developmental Programming: Prenatal Testosterone Excess Induces Hepatic Fibrosis in the Female Sheep

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Prenatal exposure to excess testosterone (T) programs peripheral insulin resistance (IR) and induces oxidative stress, ectopic lipid accumulation and IR in the liver of the female sheep. As oxidative stress and lipotoxicity can induce tissue damage leading to fibrosis with accumulation of collagen, we tested if prenatal T-treatment also increases hepatic fibrosis and if this disruption is programmed by androgen or insulin (as gestational T treatment induces maternal hyperinsulinemia and increases fetal testosterone levels). Liver tissues from control, prenatal T (100mg T propionate twice a week from days 30-90 of gestation)-, prenatal T plus androgen antagonist, flutamide (15 mg/kg/day)-, and prenatal T plus insulin sensitizer, Rosiglitazone (0.11 mg/kg/day)-treated female sheep were studied at 21 months of age, during the late follicular phase. mRNA expression of genes involved in fibrosis namely transforming growth factor beta 1 (TGFB1), fibroblast growth factor (FGF) 19 and 21, and collagen type III alpha 1 chain (COL3A1) was assessed by real-time RT-PCR and hepatic fibrosis through picrosirius red (PSR) staining for collagen. Cohen's effect size analysis found large magnitude increases in hepatic expression of TGFB1, FGF19 and COL3A1 and medium magnitude increase in FGF21 expression in the prenatal T-treated females. Barring prevention of increase in COL3A1 by androgen antagonist (large effect size), neither intervention prevented the increase in other markers of fibrosis. A large magnitude increase in hepatic collagen content was evident in prenatal T-treated females with both androgen antagonist and insulin sensitizer preventing this increase (large effect size). These findings support our hypothesis that prenatal T-treatment programs hepatic fibrosis via both androgenic and metabolic pathways. Hepatic fibrosis in the liver, likely the result of injury from oxidative stress and

ectopic lipid accumulation, may also contribute to the impaired insulin sensitivity in the liver of prenatal T-treated female sheep.
Support: NIH P01 HD44232

The Effects of Lactational High Fat Diet Exposure on Insulin and Glucose Homeostasis in Mouse Offspring

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Abstract

Background:
Offspring of obese mothers have been shown to have an increased risk of obesity. The contribution of the early postnatal period to this metabolic disease risk is not well understood.

Hypothesis:
We hypothesized that maternal high fat diet (HFD) given during the lactation period would lead to an increased obesity risk and impaired glucose homeostasis in exposed offspring.

Methods:
Adult female C57Bl6/J mice were fed a normal chow diet prior to and through pregnancy. At birth, a group of mothers were started on a 60% HFD for 3 weeks, exposing their offspring to a HFD during the lactation period (HL group). The other mothers remained on normal chow (control group). Offspring were weaned onto normal chow at 3 weeks and were challenged with a 60% HFD at 12 weeks of age. Throughout the study animals were weighed and glucose and insulin tolerance tests performed. At necropsy total pancreas insulin content and body composition were measured. Results were analyzed with one-way ANOVA.

Results:
Male offspring of the HL group had increased body weight ($p = <0.05$), whole-body adiposity ($p = <0.05$), and lean muscle ($p = <0.05$) compared to the control group. On glucose tolerance testing, male HL offspring on the HFD for 10 weeks had impaired glucose tolerance compared to controls ($p=0.021$). On insulin tolerance testing, there was no difference in insulin sensitivity between groups. The HL group had higher total pancreatic insulin content compared to controls ($p=0.015$). Female offspring had similar findings between the HL and the control group.

Conclusions:
Findings from this study indicate that maternal HFD confined to the lactation period has long term programming effects that may increase the risk for glucose intolerance and obesity in offspring. Female offspring were spared from this effect, suggesting a sex difference in lactational programming by maternal HFD.

Newborn DNA Methylation Profiles Are Associated with Neonatal Iron Deficiency and Toxicant Exposures in a Pilot Sample

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Abstract:

The *in utero* environment, including nutritional status and exposure to toxicants, may shape the offspring's epigenome, including DNA methylation, thereby influencing child development. This pilot study assessed neonatal DNA methylation changes associated with three common *in utero* conditions that adversely impact neurodevelopment – iron deficiency (ID), prenatal lead (Pb) and pesticides exposures. Epigenome-wide analysis via the Infinium HumanMethylation450 BeadChip was performed on cord blood leukocyte DNA from 36 neonates from a rural area in Zhejiang province, China. Pb levels were measured in umbilical cord blood, and ferritin and 20 pesticides were quantified in cord serum. Neonates were selected from four exposure groups: ID (n=6), high Pb (n=7), high pesticides (n=11), and control (n=10 with iron sufficiency, low Pb and pesticides). Two subjects were ID and had high Pb. Differential methylation analysis by exposure was conducted for 422,108 CpG sites using an empirical Bayes method adjusting for sex and neonatal blood cell type composition. The false discovery rate approach was used to adjust for multiple comparisons (q-values). Pb was associated with the most differential methylation ($q<0.05$), followed by ID and pesticides. For Pb, 145 CpG sites were differentially methylated; for ID there were 27. Detection of profenofos was associated with three differentially methylated CpG sites. Future plans include expanding the sample size with the long term aim to identify pathways through which adverse prenatal exposures alter the epigenome and impact neurodevelopment.

Gestational Exposure to Bisphenol A Induces Oxidative Stress in the Metabolic Tissues of the Female Sheep Offspring

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Bisphenol A (BPA) is a chemical found in common household plastics with presence detectable in drinking water, blood, urine, amniotic fluid, and breast milk. Developmental exposure to BPA is associated with reduced birth weight in humans, a risk factor for development of metabolic disorders. Gestational BPA treatment resulted in low birth weight, insulin resistance (IR) and increases in markers of oxidative stress (OS) in circulation and adipose tissue of adult female offspring. Because insulin sensitivity can be regulated by various factors including OS and muscle and liver are also major targets of insulin action, we hypothesized that prenatal BPA exposure increases markers of OS and / or decreases expression of antioxidants in insulin-target tissues. Liver, muscle and visceral adipose tissues (VAT) were collected from adult female sheep that were treated during gestational days 30-90 with none (0.0), low (0.05), medium (0.5), and high (5 mg/kg/day) doses of BPA. Markers of oxidative stress, 3-nitrotyrosine (NY) and o, o'-dityrosine (DiY), were assessed by isotope dilution liquid chromatography electrospray ionization tandem mass spectrometry and expression of antioxidants [superoxide dismutase (SOD) 1 and 2, and glutathione disulfide reductase (GSR)] by real time RT-PCR. Similar to the data reported in adipose tissue, there was an increase in NY content independent of the dose in liver and in the low and medium dose groups in the muscle. A decrease in the muscle SOD1 and 2 expression was only evident in the high BPA group. In both muscle and liver, GSR expression was reduced in the high (medium also in liver) BPA groups. In contrast, an increase in GSR mRNA was evident in the VAT of medium and high BPA groups. These findings document the increased oxidative stress status of liver and muscle but not VAT, being consistent with the insulin-resistant status of these animals.

We have shown that zebrafish (*Danio rerio*) are an ideal model for evaluating transgenerational effects of certain toxicants and their role in the fetal basis of adult disease. While the acute developmental effects of some toxicant exposures have been studied extensively, the ability of early sublethal exposures to produce adverse health outcomes in adulthood or subsequent generations is poorly understood. We used the zebrafish model to identify transgenerational effects of a known endocrine disrupting chemical (EDC), dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin; TCDD). Exposure to TCDD early in development produces reproductive abnormalities in adulthood and decreased reproductive capacity that persists for multiple, unexposed generations, and appears to be mediated through the male germline. Thus, testicular tissue from each generation was analyzed for histologic, transcriptomic and epigenetic changes. We identified significant decreases in number of spermatozoa with concurrent increase in spermatogonia, indicating delayed spermiation in TCDD-exposed males and their offspring. Gene expression analysis revealed TCDD-altered spermatogenesis, steroidogenesis, lipid metabolism, and xenobiotic metabolism pathway responses in all generations. Genes of interest include *star*, *sox9b*, *sox9a*, *vtg*, *egr1* and sf1 (*nr5a1b*). Whole-genome bisulphite sequencing identified gene-specific methylation changes associated with significantly altered gene expression in all generations. Using molecular tools in the zebrafish model, our research aims to uncover critical genes and changes in epigenetic regulation required for adverse transgenerational reproductive endpoints of EDC exposure.

Ancestral perinatal obesogen exposure results in a transgenerational thrifty phenotype in mice

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Ancestral environmental exposures to non-mutagenic agents can exert effects in unexposed descendants, adding a layer of complexity to long-standing attempts to clarify the relationships between genotypic and phenotypic variations. Transgenerational inheritance of environmental exposures has significant implications for understanding disease etiology. The environmental obesogen hypothesis proposes that exposure to obesogenic chemicals can lead to increased adiposity, *in vivo*. Here we show that exposure of F0 mice to the obesogen tributyltin (TBT) throughout pregnancy and lactation predisposes unexposed F4 male descendants to obesity when dietary fat is increased. Analyses of body fat, plasma hormone levels, and visceral white adipose tissue DNA methylome and transcriptome collectively indicate that the TBT-dependent F4 obesity is consistent with a leptin resistant, "thrifty phenotype". We found that ancestral TBT exposure induced global changes in DNA methylation together with altered expression of metabolism-relevant genes when the animals were exposed to dietary challenges. Analysis of chromatin accessibility in sperm revealed significant differences between TBT and control groups when guided by DNA sequence composition, a proxy for higher order chromatin organization. There are also significant similarities between chromatin accessibility in the sperm of F3 and F4 TBT groups that overlap with areas of differential methylation in F4 adipose tissue. Taken together, these data establish an independent connection between ancestral TBT treatment and altered chromatin accessibility that may reflect changes in higher order chromatin organization transmissible through meiosis and mitosis.

Associations of early-life social experience with DNA methylation in free-living spotted hyenas.

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Background: Rodent models and studies in captive primates indicate that early-life social experiences modulate DNA methylation patterns, which, in turn, may directly affect phenotype. Little is known of these relationships in free-living social species. In this pilot study, we examined associations between the early life social milieu, as indicated by maternal rank, and adult genome-wide DNA methylation (an indicator of genomic stability and overall health) in a population of wild spotted hyenas – a gregarious species in which maternal rank is a key determinant of access to food and resources.

Hypothesis: Higher maternal rank is associated with higher offspring genome-wide DNA methylation.

Methods: This study included 39 mother-offspring pairs in the MSU Hyena Project (n=25 female offspring, n=19 male offspring), a long-term field study of spotted hyenas in Kenya, ongoing since 1989. We determined maternal rank using a matrix of dyadic agonistic interactions recorded and updated on a yearly basis. Next, we quantified genome-wide DNA methylation in peripheral leukocytes using the LUminometric Methylation Assay (LUMA). Finally, we used multivariable linear regression to examine the relationship between quartiles of maternal rank (Q1 – highest rank; Q4 – lowest rank) and offspring genome-wide DNA methylation.

Results: Average genome-wide DNA methylation was 68.0%±11.5%. Hyenas born to highest-ranking mothers had 6.7% (95% CI: -4.8%, 18.2%) higher CCGG methylation than those born to lowest ranking mothers after accounting for sex.

Conclusions: We detected a marginally significant influence of maternal rank on offspring genome-wide DNA methylation in adulthood. These findings suggest a long-lasting impact of the early social environment on the epigenome in wild spotted hyenas. Future studies will explore this research question in a larger sample of individuals, and examine specific aspects of maternal care (e.g., nursing, grooming, food availability, peer interactions) as potential mediators of the observed relationship.

Maternal prenatal stress is associated with reduced efficiency in human fetal functional brain systems

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Emerging evidence supports a strong link between maternal prenatal stress and altered postnatal brain development. However, whether stress is reflected in brain development prior to birth, and specifically, whether maternal prenatal stress alters fetal functional brain systems, remains an open question. Given that neurodevelopmental disorders implicated in prenatal stress, such as autism and ADHD, are often characterized by decreased global neural efficiency and reduced network integration, we tested the hypothesis that higher levels of maternal stress would be associated with decreased global efficiency of the fetal neural connectome. The fetal neuroimaging sample consisted of 47 cases (28 male; age range 30-37 weeks), with mother mean age 25.1 years (SD 4.2). Spatially constrained group level clustering was used to parcellate the fetal brain into 197 spatially contiguous regions from which subject functional connectivity (FC) matrices were derived. Topological properties of FC matrices were then quantified by means of graph theoretical analyses. A measure of global neural communication efficiency was computed as the inverse of the average number of steps needed to travel from every region in the network to every other region in the network, with longer paths being less efficient. Associations between maternal stress and fetal efficiency variables were tested in a multilevel regression model that included age and motion as covariates. We discovered that higher maternal prenatal stress was associated with reduced strength of neural efficiency ($p=0.04$). Nodes in the fetal graph with the strongest effects were observed in areas of the cerebellum, medial temporal lobes, and sensorimotor regions. For the first time, we report that maternal prenatal stress exerts intrauterine programming of *in vivo* human neural functional networks. This discovery has implications for transfer of risk via early brain programming, which may be relevant to long-term psychiatric health.

Keywords: prenatal, network, stress

Funding: HHSN275201300006C (RR), MH110793 (MET), R21ES026022 (MET), R01HD075806 (MET), NARSAD (MET) T32AA007477 (RW)

Early life stress and cardiometabolic risk factor: evidence of sex-specific mechanism in rodents

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The exposure to early life stress (ELS), or adverse childhood experiences, has been proven to impact the developing organism increasing the risk for mental, immune, cardiovascular and metabolic disease. There are models in rodents to investigate the sex-specific mechanisms by which ELS is linked to chronic disease. Male rats exposed to daily maternal separation (MatSep) during postnatal life display reduced glomerular filtration, renal damage and inflammation secondary to a chronic angiotensin II (AngII) infusion. We identified increased sympathetic outflow to the kidney as a mechanism underlying heightened AngII-induced hypertension in male MatSep rats. Conversely, female MatSep rats are prone to gain weight and developing metabolic disease in response to high fat diet. We also have shown that a mouse model of ELS, called maternal separation and early weaning (MSEW), displays increased risk of developing obesity-induced hypertension. While male mice display sympathetic-activation, female mice show a greater metabolic compromise. These derangements include impaired glucose homeostasis, increased fat mass expansion, renin-angiotensin system activation and a pro-inflammatory profile. Taken together, this data indicates that “first hit” stimuli prime the response to “second hit” challenges. The use of an ELS model in rodents could provide potential therapeutic targets to reduce the cardiometabolic risk in individuals exposed to ELS.

Maternal experiences of childhood adversity are associated with age at menarche and offspring asymmetric fetal growth

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Early life psychosocial stress has a significant influence on growth and development, including sexual maturation and reproductive scheduling, with possible transgenerational effects. While early life adversity has been associated with earlier sexual maturation, it's unclear whether faster reproductive scheduling translates into differential perinatal outcomes. Early adversity can lead to risky health behaviors like smoking, and such behaviors during pregnancy may modify reproductive scheduling and birth outcome associations. This research tests whether adverse childhood experiences (ACES) predict earlier age at menarche, and whether earlier age at menarche is associated with dysregulated fetal growth. We tested whether smoking during pregnancy modified associations between ACES and fetal growth. The analysis uses data from the first 154 enrollees of the Albany Infants and Mothers Study, an ongoing prospective study of prenatal exposures and birth outcomes in Albany, New York. ACES[1] were assessed via retrospective report of traumatic events experienced by the mother before age 18. Cephalization index indicated dysregulated, asymmetric fetal growth during gestation indicative of brain sparing ((head circumference cm/birthweight g)*100))[2]. In a linear regression model adjusted for age, income during pregnancy, and self-reported race, a greater number of ACES were associated with younger age at menarche (β=-0.165, $p<0.01$). In separate linear regression models, age at menarche (β=-0.012, $p=0.09$) and maternal smoking during pregnancy (β=0.13, $p<0.01$) were associated with infant cephalization. In fully adjusted models, we observed an interactive effect between age at menarche and smoking during pregnancy (β=0.06, $p<0.01$) in relation to infant cephalization. in stratified models, the association between age at menarche and infant cephalization was positive for smokers and negative for non-smokers These results indicate that adverse childhood experiences can accelerate reproductive development for women, and that a combination of reproductive development and behaviors during pregnancy may jointly contribute to fetal growth and development.

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Maternal vitamin D depletion perturbs CpG methylation at developmental genes in the germline

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BACKGROUND: Maternal vitamin D deficiency contributes to small-for-gestation and adverse health and disease outcomes. We previously showed that gestational vitamin D depletion in mice caused heritable phenotypic changes and subtle epimutations at imprinted loci. Some epimutations were distinct between generations and not maintained from first generation sperm to second generation soma indicating correction through reprogramming.

HYPOTHESES: Maternal vitamin D depletion disrupts global CpG methylation in sperm and the epimutations are corrected in the next generation soma.

METHODS: Female Collaborative Cross CC001 mice (G_0) were treated with either AIN-93G diet (control) or modified AIN-93G lacking vitamin D (LVD). Offspring (G_1) were weaned to regular rodent chow. Treated male G_1 were bred with untreated FVB mice to generate the second generation (G_2). Bisulfite libraries were prepared from G_1 sperm using the Agilent SureSelect target enrichment system. Differentially methylated CpGs (DMCs) were identified by comparing LVD to controls using logistic regression (significance threshold, FDR<0.01). Pyrosequencing was performed to validate changes at select loci with >10% difference in methylation and are nearby development-related genes.

RESULTS: We identified global CpG methylation disruption with 16,644 DMCs evenly distributed across the genome except for enrichment on chr17 and chrX. 68% of the DMCs were hypomethylated in LVD group. Pathway analysis for genes nearby DMCs with >10% changes showed an overrepresentation for developmental pathways, suggesting affected development to alter phenotypes. Pyrosequencing validated hypomethylation at development-associated genes including tet methylcytosine dioxygenase 1 (*Tet1*), empty spiracles homeobox 2 (*Emx2*), pancreatic and duodenal homeobox 1 (*Pdx1*) and parathyroid hormone 1 receptor (*Pth1r*). These changes were not detected in G_2 9-day liver.

CONCLUSIONS: Maternal vitamin D depletion perturbs genome-wide CpG methylation in offspring germline. Fetal reprogramming potentially corrects many of the epimutations so that these changes are not maintained from G_1 germline to G_2 soma.

Integration of genome-wide methylation and gene expression data identifies epigenetically altered genes following intrauterine exposure to maternal diabetes

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Gestational diabetes mellitus (GDM) and type 2 diabetes mellitus (T2DM) complicate ~10% of pregnancies. In addition to acute risks, children of diabetic mothers are at increased risk of obesity, T2DM, and hypertension. The mechanism is not known. Our previous studies identified *PLAC8* as having altered CpG methylation and RNA expression in neonatal endothelial colony forming cells (ECFCs) obtained from the cord blood of GDM pregnancies. We hypothesize that additional genes exhibit altered epigenetic regulation following intrauterine DM exposure, as evidenced by an inverse correlation between DNA methylation and mRNA expression. Using genome-wide approaches, we aimed to discover additional genes showing such correlation following exposure to DM *in utero*. Using DNA and RNA from human ECFCs obtained from control, GDM, and T2DM pregnancies, methylation and expression was analyzed using Infinium HumanMethylation450 arrays and Affymetrix gene expression microarrays. The mean methylation value for each gene was determined for all CpG sites residing within promoter region (-2000 to +500 bases from each gene's transcription start site). Spearman correlations were performed between mean methylation value and RNA levels for each gene. Results were validated using bisulfite pyrosequencing and qRT-PCR. Ten genes showed significant correlation between DNA methylation in promoter regions and RNA expression (n=11 ECFC samples, FDR corrected $p<0.05$). The gene *DDR2* exhibited negative correlation at seven CpG sites (mean $r = -0.96$, $p<0.001$) and showed a 2.2-fold reduction in expression in DM-exposed ECFCs. Two new CpG sites near the *PLAC8* start site showing negative correlation were identified ($r = -0.68$, $p=0.005$). The discovery of multiple genes demonstrating negative correlation between DNA methylation and mRNA expression is consistent with our hypothesis that intrauterine DM exposure leads to epigenetic changes. Analysis of methylation at these sites may be instructive as biomarkers to assess effectiveness of maternal DM therapies and children's future disease risk.

Retinoid X receptor activation alters the chromatin landscape to commit mesenchymal stem cells to the adipose lineage

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Mounting evidence links developmental exposure to endocrine disrupting chemicals (EDCs) to the obesity epidemic. Many obesogenic EDCs are known to act through nuclear receptors to stimulate fat cell development. We previously showed that tributyltin (TBT) is such an EDC, acting through the peroxisome proliferator-activated receptor γ (PPARγ) and the retinoid X receptor (RXR) to bias mesenchymal stem cells (MSCs) towards the fat lineage at the expense of bone. Mice treated prenatally with environmentally-relevant levels of TBT show increased adipose depot weights, hepatic steatosis, and MSCs reprogrammed to favor the adipose lineage, effects that persist in the F1, F2, and F3 progeny of exposed F0 mothers. Importantly, undifferentiated MSCs from these mice have a pro-adipogenic gene expression profile, suggesting that TBT acts early in MSC fate specification. Current *in vitro* tools to study EDCs mechanistically during adipogenesis are limited in that they combine chemical exposure with an adipose induction cocktail, creating difficulties in distinguishing chemicals that act early during MSC lineage commitment from those that promote terminal differentiation. To overcome this limitation, we pretreated MSCs with TBT prior to differentiation with a standard cocktail. Using this new approach, we found that TBT commits MSCs to the adipose lineage in an RXR-dependent manner. Transcriptomic profiling of MSCs treated with TBT revealed genome-wide changes in gene expression that were replicated by an RXR agonist, but not a PPARγ agonist. Pathway analysis of altered transcripts indicated that TBT de-represses gene targets of Enhancer of Zeste 2 (EZH2), which deposits repressive histone 3 lysine 27 trimethyl (H3K27me3) marks on the chromatin. We found that TBT induces a genome-wide reduction and redistribution of H3K27me3 in proximity to genes that regulate adipose commitment. Our data reveal a central role for RXR in MSC lineage commitment and raise the stakes for identifying other EDCs that activate RXR.

In utero exposure to bisphenol A alters genome wide DNA methylation and gene expression in human fetal stem cells

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ABSTRACT:

Environmental toxicants that alter the intrauterine milieu can affect fetal development by modifying gene function of pluripotent cells that are rapidly replicating. During fetal development, the rapidly replicating pancreatic beta-cells, hepatocytes, myocytes and adipocytes are particularly susceptible to exposure to environmental toxicants and their altered function contributes to the development of adult onset metabolic disorders such as diabetes, obesity and non-alcoholic fatty liver disease (NAFLD). Previously we have shown that prenatal exposure to the endocrine disrupting chemical bisphenol A (BPA) is associated with lower birth weight in humans and epidemiological and animal studies report that low birth weight is associated with obesity and diabetes later in life. Therefore, **we hypothesized that *in utero* exposure to BPA alters genome wide DNA methylation and gene expression in fetal stem cells at metabolically relevant genes.** We measured changes in gene expression via RNA-Seq and genome wide DNA methylation (via ERRBS) in human amniocytes, a fetal derived stem cell, exposed to BPA *in utero* assessed from second trimester amniotic fluid total BPA concentration (2.57±1.84 ng/mL). We performed separate analyses to identify differentially methylated regions (DMRs) on amniocytes exposed to BPA. We identified 9 DMRs when amniocytes of both sexes were analyzed as a group (n=20) and 199 and 46 DMRs in the male and female amniocyte specific analyses, respectively (n=10) ($p<0.05$). In the RNA-Seq differential expression analysis, we identified 3 genes when analyzing all amniocytes together (n=6) but 43 genes in the male amniocyte analysis (n=3) ($q<0.05$). BPA exposure resulted in enrichment of genetic pathways including Notch signaling, NAD Redox metabolism, PPAR signaling, hepatic stellate cell activation, cardiomyocyte differentiation and thyroid cancer signaling.

Mice Lacking Membrane-Localized Estrogen Receptor 1 Exhibit Contrasting Uterine and Vaginal Responses to Neonatal Estrogen Chemical Exposure

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Endocrine disrupting chemicals (EDCs), such as diethylstilbestrol (DES, a potent xenoestrogen) bind and activate both estrogen receptor 1 and 2 (ESR1 and ESR2). Effects of EDCs on estrogen receptors have been focused primarily on nuclear forms of such receptors. However, a small proportion of ESR1 is found in cellular membranes following post-translational palmitoylation. Binding of estrogens to membrane ESR1 (mESR1) activate protein kinases\. Herein, nuclear-only ESR1 (NOER) mouse model was used to determine whether neonatal exposure to DES followed by adult exposure to 17β-estradiol (E2) affected uterine and vaginal cell proliferation and uterine gene expression. Ablation of mESR1 might confer partial protection against adverse effects of DES and E2 exposure. Pups were treated subcutaneously with 1mg DES/kg BW from postnatal day 1-5. At day 60, females were ovariectomized, and 14 days later, injected with vehicle or E2 (10µg/kg BW, i.p.). Twenty-four-hours later, uterine and vaginal samples were fixed in 10% neutral buffered formalin or frozen in liquid N_2 (n = 2-4 per group). Formalin-fixed-tissues were processed and immunostained with a cell proliferative marker, MKI67, and percentage of cells staining positive quantified. RNA was isolated from uteri, and gene expression analysis is underway for several steroid receptor and epigenetic-associated genes. Preliminary uterine histological results indicates number of MKI67+ glandular epithelial, stromal, and total cells was greater in WT animals exposed to DES and E2 relative to estrogen exposed NOER mice (P value range = 0.001 to 0.01). Conversely, data suggests WT mice exposed to both estrogens had less positive staining vaginal epithelial cells compared to estrogen-treated NOER mice (104.5 ± 27.3 vs. 208.0 ± 23.6, respectively; P = 0.01). In summary, NOER mice showed a differential proliferative response to estrogens relative to WT counterparts, but this response varies according to reproductive organ examined. Future studies might provide insight into underpinning mechanisms.

Associations of weight- and length-derived anthropometric indicators with body composition at birth and 5 months: The Healthy Start Study

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Background: Little is known regarding the extent to which weight- and length-based anthropometry indices correspond with fat vs. lean mass during infancy. We examined associations of weight-for-age (WFAZ), weight-for-length (WFLZ), and body mass index (BMIZ) z-scores with fat mass (FM), % fat mass (%FM), and fat-free mass (FFM) measured by air-displacement plethysmography during the first 5 months of life.

Hypothesis: BMIZ and change (Δ) in BMIZ is the most suitable proxy for infant fat mass and adiposity gain.

Design: Using data from 1,027 infants in the Healthy Start Study, we used multivariate regression to evaluate relationships of the z-score indicators with body composition at birth, 5 months, and Δ during follow-up. Additionally, we directly compared the utility of Δ in each z-score indicator as a proxy of adiposity gain according to Δ%FM using multivariate analysis of variance (MANOVA).

Results: At birth, all three indicators were more strongly associated with FFM than FM. Each unit of WFAZ corresponded with 0.342 (95% CI: 0.331, 0.351) kg FFM, vs. 0.121 (0.114, 0.128) kg FM ($p<0.0001$), with similar trends observed for WFLZ and BMIZ. By 5 months, WFLZ and BMIZ were more strongly associated with FM than FFM, whereas WFAZ was similarly related to FM and FFM. Change in WFLZ and BMIZ were both more strongly related to ΔFM than ΔFFM; however, a direct comparison of all three indicators via MANOVA revealed that Δ%FM was most strongly associated with ΔBMIZ. Each unit increment in Δ%FM corresponded with 0.110 (0.099, 0.122) units ΔBMIZ, as compared to 0.091 (0.082, 0.101) units ΔWFAZ ($p<0.0001$), and 0.097 (0.085, 0.110) units ΔWFLZ ($p<0.0001$).

Conclusions: Weight- and length-based indices are poor surrogates for newborn adiposity. At 5 months, WFLZ and BMIZ are suitable proxies. Change in BMIZ is the best indicator of fat accrual during the first 5 postnatal months.

Maternal Engineered Nanomaterial Inhalation Increases Cardiovascular Disease Susceptibility in Young Offspring

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Cardiovascular disease (CVD) is the leading cause of death worldwide and associated with many genetic and lifestyle risk factors. The fetal milieu during gestation has been explored as a contributing factor to progeny health. Engineered nanomaterials (ENM) are manufactured within the nanoscale (<100 nm in one dimension) to take advantage of specific physiochemical properties. Exposure to these materials has become more prolific given their pervasiveness in domestic applications. While initial occupational studies have identified toxicities, the outcomes during pregnancy and for developing young are unknown. Gestational ENM exposure led to impairments in uterine microvascular reactivity in early studies, indicative of the development of a hostile gestational environment.

Microvascular dysfunction has been established as an early mark of CVD. Therefore, studies were initiated to assess the microvascular health during growth from a late-stage fetus to young adult. Pregnant dams were exposed to nano-sized titanium dioxide aerosols (10.4±0.1 mg/m^3 for 4h, aerodynamic diameter of 136.5±1.4 nm, calculated daily pulmonary deposition of 39.7±1 µg, initiated on gestational day [GD] 5.78±0.13 for 7.78±0.26 days of the remaining pregnancy). Microvascular reactivity was assessed via a pressurized isolated microvessel preparation wither the arterioles of the tail (fetal [GD 20], neonate [6±0.48d] or heart (weanling [25.7±0.9d], juvenile [7.64±0.4w], adult [10.36±0.34w]) progeny were dissected, excised, and evaluated in response to chemical stimuli to calculate endothelium-dependent (acetylcholine [10^{-9}-10^{-4} M]), -independent (spermine-NONOate [10^{-9}-10^{-4} M]), and vascular smooth muscle (phenylephrine [10^{-9}-10^{-4} M]) function. Significant temporal impairments in endothelium-dependent reactivity were identified in exposed fetal (-37.2% ±7.3), juvenile (-29.1% ±11.3), and adult (-29.1% ±11.3 vs. control, respectively) progeny.

Our previous work has evaluated maternal inflammation, mitochondrial, oxidative, and epigenetic mechanisms in the progeny after maternal ENM inhalation. Exposure to ENM during gestation may increase CVD susceptibility, thereby compromising health of future generations.

NIH-R01-ES024783 (PAS); P30-ES005022

ABSTRACT

Background: Childhood diet may play a role in the timing of sexual maturation; although previous research has not evaluated whether dietary patterns, which take into account correlation of foods, relate prospectively to puberty. Diet patterns during the critical period surrounding the adiposity rebound may be particularly relevant.

Hypothesis: We hypothesized that diet patterns at 3 years (y) of age would relate to pubertal timing in a Mexican population.

Methods: The sample population included 496 children from sequentially-enrolled birth cohorts from Mexico City. Mothers completed an interviewer-administered semi-quantitative food frequency questionnaire when the child was 3 y of age, and a physician assessed sexual maturation Tanner stages at a visit between the ages of 9 and 18 y. Girls were asked if and when menarche had occurred. We used principal component analysis to identify diet patterns at age 3 y. We estimated hazard ratios and 95% confidence intervals (CI) for having reached Tanner stage ≥4 or testicular volume ≥15 by the follow-up visit or the time to menarche, according to dietary pattern tertile.

Results: We identified 5 dietary patterns at age 3 y that explained 30.3% of the variance. Among girls, those in the highest tertile of *vegetables and lean proteins* pattern scores had a 35% lower probability of having reached breast stage ≥4 at the follow-up visit compared to the first after confounder adjustment (95% CI 3% to 67%; P, trend=0.03). Among boys, those in the highest tertile of *processed meat and refined grain* pattern scores had a 3.58 times higher probability of having reached testicular volume ≥15 than boys in the lowest tertile after confounder adjustment (95% CI 0.62 to 6.53; P, trend=0.02).

Conclusions: Diet patterns at 3 y of age were related to timing of puberty in a sex-specific manner among Mexican children.

Does paternal bisphenol A exposure affect offspring glucose tolerance? A peek through exposure windows reveals that timing is of the essence.

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ABSTRACT

As it is becoming increasingly accepted that maternal exposure to the ubiquitous environmental pollutant bisphenol A (BPA) predisposes offspring to metabolic impairments, paternal contribution in this context remains unresolved. To investigate a relationship between paternal BPA exposure and offspring obesity and glucose tolerance, an accepted experimental mouse model was employed using dietary BPA exposure at doses comparable to human exposure levels. These doses were 7% Corn Oil diet (Control), 10 µg/mg/day (Low BPA), and 10 mg/kg/day (High BPA). Two exposure windows were investigated: 1) exposure of male mice beginning at sexual maturation (5 weeks of age) and continuing for 12 weeks prior to mating, and 2) *in utero* exposure, in which dams were exposed beginning 2 weeks prior to mating and ending at weaning. The male offspring of the exposed dams were regarded as *in utero*-exposed F0 sires. All F0 sires were mated to unexposed females. At 16 weeks of age, F1 offspring from both exposure windows underwent body composition analysis via NMR or DEXA scan as well as glucose tolerance testing. Paternal BPA exposure during adulthood did not affect body weight, adiposity or glucose tolerance in offspring. However, *in utero*-exposed sires produced female offspring with impaired glucose tolerance. Body weight and adiposity were unaffected in either sex. These data demonstrate that while paternal BPA exposure after sexual maturity may be metabolically innocuous, paternal BPA exposure during gestation and lactation precipitates sex-specific impairments in glucose tolerance. Further studies are required to describe precisely the glucose homeostatic impairment and elucidate epigenetic changes in sperm associated with phenotypic transmission.

BPA Concentrations in Liquid Supernatants of Imported Canned Foods quantified by Sensitive BPA ELISA

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BPA [2,2-(4,4'-dihydroxydiphenyl)propane], an endocrine disruptor, increases the risk of early puberty, infertility, breast and prostate cancers and insulin resistance. A source of dietary BPA is BPA leached from epoxy film-coated cans. A recent study with LC/MS/MS found BPA levels in canned food solids of 16 different food types ranged from 0 to 730 ng/g with large variations among different products of the same food type and even different lots of the same product. A sensitive and facile BPA ELISA has been developed to monitor BPA concentrations in liquid supernatants of various canned foods. BPA antibodies were produced by immunization of a goat with carboxylalkyl-derivatized BPA conjugated to KLH. Anti-BPA did not cross-react with BPF, less frequently used than BPA for epoxy film production. BPF contains two phenol groups as in BPA, but lacks dimethyl groups at the carbon bridge. Anti-BPA did not cross-react with resveratrol, which contains single phenol group. Liquids drained from imported canned foods (6 kinds, 2 kinds/country) were centrifuged and BPA concentrations of the supernatants were measured after 10 to 100-fold dilution with tris-buffered saline (TBS) using BPA ELISA. Among supernatants obtained from the 6 products (3 cans/each kind, 9 data points), 3 products contained 6.83 ± 0.76 ng/ml, 8.50 ± 0.87 ng/ml and 11.83 ± 0.29 ng/ml of BPA (first group), similar to ~10 ng/ml of BPA levels in supernatants from national canned soups. However, 3 products contained 98.67 ± 3.22 ng/ml, 185.00 ± 13.23 ng/ml and 193.33 ± 11.55 ng/ml of BPA (second group), ~11 to 21-fold higher than the mean value of the first group. These results demonstrate that liquid supernatants from a significant number of imported canned foods have BPA levels higher than those of national

canned soups and the BPA ELISA is suitable for high-throughput screening (HTS) of the liquid supernatants.

Title: Impact of non-heme brain iron and age on cognitive ability during childhood and adolescence

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Abstract body word count: 293

Background: Non-heme iron is a vital metabolic cofactor for core processes of brain development, including myelination, dendritogenesis, and neurotransmitter synthesis, and accumulates in the brain with age. However, less is known about age-related changes in iron, and their association with emerging cognitive abilities during formative years.

Hypothesis: Based on post-mortem and MR studies of lifespan iron accumulation, we hypothesized that iron levels would increase with age, and that both iron levels and the magnitude of age-related increases would vary by region. Given the critical role of iron in brain development, we predicted that greater iron would relate to better performance on cognitive measures.

Methods: In this study, we examined non-heme iron content in basal ganglia substructures and the hippocampus using susceptibility weighted imaging (SWI) in a sample of fifty-seven children, ages 7-16. Non-heme iron signal was extracted from the center of each ROI. Cognitive ability was assessed by raw IQ scores and processing speed. Data reduction was used to minimize the number of multiple comparisons. General linear modeling was performed for iron content, age (mean-centered) and sex predicting differences in processing speed and general intelligence.

Results: We find that regions differed in gross iron content, $F(5,50) = 13.28$, $p < 0.001$, but hemispheres did not. Age correlated positively with iron content across regions, $F(1,54) = 47.22$, $p < 0.001$, but the magnitude of age differences in iron content differed between regions, $F(5,50) = 5.50$, $p < 0.001$. Controlling for age, greater brain iron content accounted for faster processing speed ($F(1,55) = 5.96$, $p = 0.02$; BS 95% CI: 0.10/0.53) and better general IQ ($F(1,55) = 7.92$, $p = 0.01$; BS 95% CI: 0.09/0.71).

Conclusion: These findings support non-heme iron's critical neurobiological role in the development of cognitive abilities during childhood with advancing age.

Title: Cord blood buffy coat DNA methylation is comparable to whole cord blood methylation

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Abstract:
Background: Cord blood DNA methylation is associated with numerous health outcomes and environmental exposures. Whole cord blood DNA reflects all nucleated blood cell types, while centrifuging whole blood separates red blood cells by generating a white blood cell buffy coat. Both sample types are used in DNA methylation studies. Cell types have unique methylation patterns and processing can impact cell distributions, which may influence comparability.

Objectives: To evaluate differences in cell composition and DNA methylation between buffy coat and whole cord blood samples.

Methods: Cord blood DNA methylation was measured with the Infinium EPIC BeadChip (Illumina) in 8 individuals, each contributing buffy coat and whole blood samples. We analyzed principal components (PC) of methylation, performed hierarchical clustering, and computed correlations of mean-centered methylation between pairs. We conducted moderated t-tests on single sites and estimated cell composition from methylation.

Results: DNA methylation PCs were associated with individual ($P_{PC1}=1.4x10^{-9}$; $P_{PC2}=2.9x10^{-5}$; $P_{PC3}=3.8x10^{-5}$; $P_{PC4}=4.2x10^{-6}$; $P_{PC5}=9.9x10^{-13}$), and not with sample type ($P_{PC1-5}>0.7$). Samples hierarchically clustered by individual. Pearson correlations of mean-centered methylation between paired individual samples ranged from r=0.66 to r=0.87. No individual site significantly differed between buffy coat and whole cord blood when adjusting for multiple comparisons (5 sites had unadjusted $P<10^{-5}$). Estimated cell type proportions did not differ by sample type (P=0.86), and estimated cell counts were highly correlated between paired samples (r=0.99).

Conclusions: Differences in methylation and cell composition between buffy coat and whole cord blood are much lower than inter-individual variation, demonstrating that both sample preparation types can be analytically combined and compared.

Thermogenesis and Uncoupling Protein 1 in the Mouse Model of the Developmental Programming of Metabolic Syndrome - Preliminary Investigations

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BACKGROUND:
Epidemiological and experimental data demonstrate that offspring develop a metabolic syndrome if their mothers were obese during prenatal period. With increasing interest in brown adipose tissue (BAT) as a possible therapeutic target to counteract obesity and insulin resistance, in utero environment could represent a critical window to possibly modify BAT function and browning of white adipose tissue.
HYPOTHESIS:
Thermogenesis and levels of Uncoupling Protein 1 (UCP1) are altered in offspring exposed to prenatal obesity.
METHODS:
Study Design: Female CD1 mice were fed either high fat diet (34.9% fat, HF group) or regular chow (5.8% fat, SF group) for 3 months before breeding. After weaning, all pups were placed on a regular diet. At 6 months of age, brown (BAT), subcutaneous (SAT) and visceral (VAT) adipose tissues were collected. Total (TR) and UCP1-dependent (UCP1-R) mitochondrial respiration as proxy to thermogenesis was assessed using high-resolution respirometry. UCP1 mRNA and protein levels were quantified using q RT-PCR and Western blot analysis, respectively. Student's t-test and Mann-Whitney test were used as appropriate (significance: $P<0.05$)
RESULTS:
TR, UCP1-R and UCP1 protein levels were significantly higher in BAT from HF males ($P=0.005$, $P=0.02$, $P=0.04$, respectively) and HF females ($P=0.02$, $P=0.01$, $P=0.04$, respectively) when compared to SF.
In SAT, the UCP1-R was significantly lower from HF males ($P=0.04$) while UCP1 protein levels were significantly lower in HF females ($P=0.03$) with no differences in TR between HF and SF groups.
And in VAT, UCP1-R and UCP1 protein levels were significantly lower in HF females ($P=0.04$, $P= 0.03$, respectively), with no differences between male groups. No differences in TR between HF and SF groups were determined in VAT.
CONCLUSIONS:
Our pilot findings support a hypothesis that prenatal exposure to obesity leads to modification in BAT and browning of WAT. Moreover, these alterations are different in males and females.

US DOHaD 2017 Abstract

The Relationship between Maternal Weight and the Fetal Autonomic Nervous System

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Background: Fetal heart rate variability (HRV) is an index of the fetal autonomic nervous system that is associated with neurodevelopmental outcomes in the infant. Maternal obesity is an established risk factor for poor infant neurodevelopmental outcomes; however the link between fetal HRV and maternal weight throughout pregnancy is unknown. We hypothesized that maternal obesity may negatively impact fetal autonomic nervous system development, thereby elucidating a possible mechanism initiated *in utero* leading to poor developmental outcomes among infants born to obese mothers.

Methods: Maternal-fetal magnetocardiograms were recorded using an 83-channel dedicated fetal biomagnetometer at 36 weeks gestation (n=46). Maternal and fetal cardiac components were separated using independent components analysis. Fetal HRV was represented by the standard deviation of sinus beat-to-beat (NN) intervals (SDNN). Maternal weight was measured at enrollment (between 12 and 20 weeks gestation), 36 weeks, and delivery. Bivariate (Pearson) correlations were determined between weight at each time point and fetal SDNN at 36 weeks.

Results: Maternal weight at all time points was negatively associated with fetal heart rate variability at 36 weeks, such that women with a higher weight at enrollment (r=-0.387, p=0.008), 36 weeks (r=-0.380, p=0.009), and delivery (r=0-0.427, p=0.003) had lower fetal HRV.

Conclusions: We observed a clear and consistent relationship between maternal weight and fetal heart rate variability. Interventions are needed to determine if a reduction in maternal obesity could lead to improved fetal autonomic cardiac control.

Postpartum maternal fat distribution and its association with offspring body fat through the first year of life.

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Background: Maternal obesity is known to increase the risk of offspring obesity. Despite the evidence supporting the impact of maternal obesity on infant health, there are no studies examining the effects of maternal fat distribution on the programming of offspring obesity.

Hypothesis: We hypothesized that increased maternal visceral fat would be associated with increased infant body fat at 2 weeks, 6 months, and 12 months of age.

Methods: Participants were mother-child dyads enrolled in a longitudinal study assessing the effects of maternal obesity on infant health. Mothers were separated into either the normal weight (n=85) or overweight/obese (n=103) group based on early pregnancy BMI. Maternal body composition was assessed 4 weeks postnatally using dual-energy X-ray absorptiometry and infant body composition was assessed at 2 weeks, 6 months, and 12 months of age using nuclear magnetic resonance imaging. Linear mixed-effects models were used to investigate the association between the infant's percent body fat during the first year of life and maternal adiposity distribution, accounting for maternal race, age, first trimester activity, energy expenditure, respiratory exchange ratio, gestational age and weight gain, delivery method, infant's birth weight, birth length, sex, and length of breastfeeding.

Results: Normal weight mothers had significantly lower BMI, total body fat, subcutaneous fat, and visceral fat than overweight/obese mothers ($p<0.0001$). As a continuous variable, maternal

total body fat and subcutaneous fat were positively associated with increased infant body fat at 2 weeks ($p<0.01$), 6 months ($p<0.01$), and over the first year of life ($p<0.01$). Maternal visceral fat was not associated with increased infant body fat in the longitudinal model ($p=0.71$).

Conclusions: This study demonstrated that postpartum visceral fat did not associate with offspring fat mass. However, greater postpartum total body fat and subcutaneous fat predicted increased offspring fat mass through the infant's first year of life.

Maternal high-fat diet modulates brown adipose tissue response to β-adrenergic agonist

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Maternal obesity increases offspring risk for several metabolic diseases. We previously showed that offspring of obese dams are predisposed to obesity, liver and adipose tissue anomalies. However, the effect of maternal obesity on developmental programing brown adipose tissue (BAT) is poorly understood. By using a mouse model of HFD-induced obesity, we investigated changes in BAT following β3-adrenergic receptor (β3AR) agonist, CL-316243 treatment. Female C57BL6/J dams were fed either control (17% fat) or HFD (45% fat) and bred with lean male mice. Following weaning, offspring from both diet groups remained on control diets (CC, HC respectively). At 20 weeks of age, offspring were treated with vehicle or β3-adrenergic receptor agonist CL-316243 (CL, 1 mg/kg i.p. for one week, n=4-5). H&E staining revealed larger adipocytes and more lipid deposition in BAT of offspring of HFD dams. Global RNA transcriptomic analysis revealed distinct effect of maternal HFD feeding. Principal component analysis revealed clustering of samples based on the maternal diet. Maternal HFD altered expression of 89 genes (±2-fold change, adj $p<0.05$). Enrichment of gene ontology biological processes revealed genes related to cellular metabolic process, response to stress, chemical, temperature stimulus and heat. Following CL-316243 treatment HC offspring showed impaired physiological responses. Classical BAT specific proteins such as Ucp1, Dio2 and Cidea were downregulated in BAT of HC offspring upon CL-316243 challenge. In addition, protein kinases such as AMPK as well as ERK1/2 were also down regulated in HC groups treated with CL-316243. In conclusion, our results indicate that maternal obesity programs adipose tissue response to the β3AR stimulation which may influence pathways regulating energy expenditure, metabolism and overall risk of obesity. These studies were supported by USDA CRIS 6206-51000-010-05S.

High Fat Diet Abolish the Development of Endothelial Dysfunction in Female Rats Exposed to Maternal Separation

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Metabolic dysfunction is known to induce detrimental effects on vascular function. We have shown that female rats exposed to maternal separation (MatSep), a model of early life stress, display increased fat mass and impaired glucose metabolism in response to a high fat diet (HFD). These effects were reverted via the postnatal treatment with metyrapone (MTP), a glucocorticoid synthase inhibitor. This study investigated whether MatSep superimposed to a HFD will worsen vascular reactivity and endothelial function in thoracic aortic rings. MatSep was performed in female pups 3-hr/day from postnatal days 2-14. Normally-reared littermates served as controls. Rats were kept on either a normal diet (ND,18%kcal-fat) or placed on a HFD (60%kcal-fat) for 12 weeks. Thoracic aorta was excised to perform wire myography dose-response curves. MatSep attenuated phenylephrine (PE)- and Angiotensin II (AngII)-induced vasoconstriction in aortic rings from rats fed a ND ($p<0.05$); however, HFD increased these responses in both groups similarly. HFD downregulated α1-adrenergic receptor's gene expression while increased IL-6 and TNFa mRNA abundance in aorta from MatSep rats vs. C ($p<0.05$). Aortic endothelial function was impaired by MatSep in rats fed a ND and associated with reduced nitric oxide synthase (NOS3) mRNA expression ($p<0.05$). Remarkably, HFD significantly improved acethylcholine-induced relaxation only in MatSep rats. This improvement was accompanied by a increase in NOS3 mRNA expression ($p<0.05$). A separate group of pups was treated with MTP (50 µg/g/day, postnatal days 2-14) and weaned on a HFD. The MTP treatment attenuated PE and AngII-induced constriction similarly in both MatSep and control rats fed a HFD. Thus, these data indicates that increased vascular constriction in response to a HFD is associated to a glucocorticoid-dependent mechanism. However, despite increases in adiposity and glucose intolerance, female MatSep rats fed a HFD display preserved endothelial function that could be mediated via glucocorticoid-induced NOS3 overexpression.

Prenatal phthalate metabolite concentrations and infant fat mass across the first year of life: a pilot study

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Background: Phthalates are endocrine-disrupting chemicals associated with childhood obesity. While studies have found positive associations for certain prenatal and postnatal urinary phthalate metabolites and weight/length or BMI as indicators of adiposity levels, little is known about the direct associations between phthalates and fat accretion in early life.
Hypothesis: Prenatal urinary phthalate metabolite concentrations are associated with infant fat mass (FM) measured by quantitative magnetic resonance (QMR) across 12-months.
Methods: Using a prospective design, a total of 20 white mothers were included based on their pre-pregnancy body mass index (BMI) and FM (n=10 obese: $\geq$30kg/m^{2}and $\geq$30% FM; n=10 normal weight: <25kg/m^2 and <30% FM). Urine was collected at 30 weeks gestation and analyzed for 9 urinary phthalate metabolites and divided at the median. Children's FM were obtained at 2-weeks, 6-months, and 12-months of age using QMR. ANOVA and generalized linear models evaluated the associations between prenatal phthalate metabolite concentrations and infant FM measures, while adjusting for potential confounders (maternal BMI, FM, and gestational weight gain).
Results: Higher than median concentrations of mono-(3-carboxypropyl) phthalate (MCPP) was associated with higher infant FM at 6-months and 12-months of age ($p<0.03$). Higher mono-ethyl phthalate (MEP) concentrations were associated with lower infant FM at 12 months ($p=0.008$). After adjusting for confounders, infants born to mothers with $\geq$ median MCPP levels had a 4.2% higher FM at 12-months compared to infants born to mothers with <median MCPP levels (95% CI: 0.1, 8.2). Associations for 6-month FM were no longer significant after adjustment for confounders. Higher than median MEP levels were associated with -5.9% lower FM at 12-months compared to infants born to mothers with <median MEP levels (95% CI: -9.0, -2.7).
Conclusion: These findings suggest a potential association between certain prenatal phthalates exposure and infant FM during the first year of life.

Background: Analysis of human placenta for toxic metals/metalloids is gaining increasing interest in children's biomonitoring studies as it potentially provides information on fetal exposure during critical windows of development.
Hypothesis: We examine the association between the Pb content of placenta (body and membrane) and umbilical cord, with the infant's blood lead (BPb) level at birth, 6 and 12 months.
Methods: The Albany Pregnancy Infancy Lead Study sample consists of lower income women living in Albany County, NY and delivering at Albany Medical Center Hospital between 1992 and 1997. Placentas were collected at birth, frozen and analyzed for Pb at the New York State Department of Health's Wadsworth Center, using methods based on inorganic mass spectrometry (ICP-MS). In addition to cord blood, venous blood was collected at 6 and 12 months of age and analyzed for Pb using graphite furnace atomic absorption (GFAAS). Mother's BPb was assessed at birth and in 2^{nd} and 3^{rd} trimesters. Infant's development at 12 months was assessed using the Bailey Scale of Infant Development.
Results: Placental body Pb is significantly correlated ($p<0.001$) with infants' BPb at birth ($r=0.314$), at 6 months ($r=0.271$) and 12 months ($r=0.278$). Umbilical cord Pb was similarly associated with infant's BPb. Only placental membrane Pb predicted infant's BPb level at birth more strongly ($r=0.546$) but was more weakly associated with BPb at 6 and 12 months. Bivariate correlations between placental Pb and infant's Bailey scores were weak. Only the umbilical cord Pb was significantly associated with Bailey score ($r=0.18$, $p=0.05$). In multivariate models with covariates (birth weight, mother's WAIS, education level, gestation age, HOME score) none of the placental measures was significantly associated with infant's Bailey score.
Conclusion: In this population, placental Pb was moderately associated with infant's BPb levels but not with the infant's score on the Bailey scale of development.

TITLE: The effect of developmental neuronal iron deficiency on DNA hydroxymethylation
AUTHORS: Amanda Barks, Phu Tran, Michael Georgieff

Background: Iron deficiency (ID) during neurodevelopment results in lasting neurocognitive deficits and emotional-behavioral abnormalities. In animal models, developmental ID also causes permanent alterations in hippocampal gene expression that last into adulthood despite early-life iron repletion. Thus, early life ID likely developmentally reprograms hippocampal gene regulation. Epigenetics is a potential mechanism underlying such developmental programming. The epigenetic modification 5-hydroxymethylcytosine (5hmC) is generated by Ten-Eleven-Translocation (TET) proteins. Importantly, TET's catalytic activity requires iron, thereby representing a potential iron-dependent epigenetic mechanism in neural tissue.

Hypothesis: Acute neuronal iron deficiency decreases global 5hmC levels, mediated by decreased TET enzymatic activity.

Methods: HT22 cells, an immortalized mouse hippocampal cell line, were cultured. 24-hrs after plating, cells were treated with 10uM deferoxamine (DFO), an iron chelator, or vehicle control. RNA and genomic DNA were collected from DFO-treated and vehicle-treated (control) cultures 24-hrs after treatment. Transferrin receptor-1 (Tfrc) expression was assessed by RT-qPCR to index cellular iron deficiency. 5hmC was quantified using the MethylFlash Hydroxymethylated DNA Quantification Kit (Epigentek).

Results: 10uM DFO resulted in 2.54-fold upregulation of Tfrc compared to controls ($p<0.0001$), indicating that cellular ID was successfully induced in cultured cells. Global 5hmC will be quantified and compared between DFO-treated and control cultures.

Conclusions: Epigenetic modifications may play a role in developmental programming of hippocampal gene expression changes following early-life ID. 5hmC is iron-dependent, and positively correlates with gene expression, particularly in the central nervous system. Thus, it presents a possible epigenetic mechanism, by which developmental ID permanently alters hippocampal gene regulation.

The industrial by-product TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is a potent environmental toxicant and endocrine-disrupting chemical (EDC) with known teratogenic effects on humans, rodents and fish. Developmental exposure to some EDCs, including TCDD, is linked to the occurrence of adult-onset and multigenerational disease. Our lab uses zebrafish (*Danio rerio*) as a model to study these effects due to their short generation time, transparency in early development, and ease of developmental exposure. Previous work in this lab has shown that both structural and reproductive abnormalities (spinal deformities, sex ratio skewed toward female fish, body plan/gonad mismatch, and decreased fertility) were observed in young zebrafish exposed to TCDD. Reproductive abnormalities observed in subsequent unexposed generations (F_1 and F_2) were male-mediated, which suggested heritability through the male germline. We analyzed the testicular tissue of TCDD-exposed male zebrafish from all three generations, looking for changes in histology and gene expression that could account for decreased reproductive capacity. For histological analysis, spermatogenic cells were categorized by differentiation stage and quantified within seminiferous tubules. Statistical analysis (Student's t-test) demonstrated significant differences in certain spermatogenic cell types between exposed and control groups in the F_0 and F_1 generations, indicating delayed spermiation in exposed males and descendants. Analysis of exposed testes revealed multigenerational gene expression changes in pathways implicated in reproduction and infertility, including testis development and spermatogenesis, lipid metabolism and steroidogenesis, citric acid cycle, peroxisome, and aryl hydrocarbon receptor (AhR) xenobiotic response pathways. Overall, we found that differential expression of reproductive genes and reduced capacity of sperm cells to mature could account for the reproductive defects previously seen in TCDD-exposed male zebrafish and their descendants.

Title: CAN HONOKIOL BE USED TO AMELIORATE PREECLAMPSIA?

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ABSTRACT:

Epoxyeicosatrienoic acids (EETs) have antihypertensive properties and the attenuation of their conversion to dihydroxyeicosatrienoic acids (DHETs) by inhibiting the activity of soluble epoxide hydrolase (sEH) has been tested as a therapeutic approach for treating hypertension. Kidney cells have a relatively high sEH activity and decreased renal EET levels have been linked to hypertension. The aims were to study the involvement of sEH activity in preeclampsia by the measurement of urinary 14,15-DHET levels and to test whether use of honokiol (a natural component of magnolia bark) inhibits sEH activity. Urine samples from healthy and preeclamptic pregnant women (blood pressure of 120±8/72±7 and 152±13/95±7 mmHg, respectively) were collected and incubated with or without glucuronidase to enable the measurement of the glucuronidated and free forms. For the cell culture experiments, human kidney endothelial cells (ACHN) were treated with AUDA, a known synthetic sEH inhibitor, or of honokiol for 2-hours followed by the addition of various concentrations of EET (0.3, 1 and 5 µM) to the cell media. Also, the effect of AUDA and honokiol on enzyme activity was tested using sEH recombinant enzyme. Concentration of 14,15-DHET in human urine and cell media was assessed using 14,15-DHET ELISA. Levels of total (glucuronidated + free) 14,15-DHET were higher in urine samples obtained from preeclamptic women compared to healthy pregnant women (100±20 vs 38±12 ng 14,15-DHET/mg creatinine). In kidney endothelial cells, either honokiol or AUDA decreased the formation of 14,15-DHET at 0.3, 1 and 5 µM concentrations of EET and treatment of cells with a mixture of both compounds showed a minimal additive effect. The increased urinary 14,15-DHET levels in pregnant women strongly suggest that sEH is involved in preeclampsia. Moreover, the

inhibitor study suggested that honokiol consumption might be effective in decreasing sEH activity and maintaining renal EET levels, thus, preventing the onset of hypertension.