DIABETES

Abstracts 62 – 70

Moderators: Mark Landon, MD; Kim Boggess, MD

62 Hyperglycemia impairs cytotrophoblast function via stress signaling

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OBJECTIVE: Diabetes mellitus is a risk factor for preeclampsia (preE). The normal extent of cytotrophoblast (CTB) invasion of the decidua, which is facilitated by plasmin, may be inhibited in preE. Inactive plasminogen is converted to plasmin by urokinase plasminogen activator (uPA), and uPA is regulated by plasminogen activator inhibitor 1 (PAI-1). Peroxisome proliferator-activated receptor gamma (PPARγ) also is implicated in the dysfunction of CTBs in hyperglycemic conditions. This study assesses the signaling mechanisms of hyperglycemia-induced CTB dysfunction.

STUDY DESIGN: Human CTBs (Sw. 71) were treated with 45, 135, 225, 495 or 945 mg/dL glucose for 48h. Some cells were pretreated with a p38 inhibitor (SB203580) or a PPARγ ligand (rosiglitazone). Thereafter, cell lysates were utilized to measure uPA, PAI-1 and PPARγ expression and p38 Mitogen Activated Protein Kinase (MAPK) phosphorylation by western blot. The mRNA expression of uPA and PAI-1 in CTB lysates was measured by qPCR. Levels of angiogenic (soluble fms-like tyrosine kinase-1 (sFLT-1), soluble endoglin (sENG)) and anti-angiogenic factors (VEGF, PlGF) and IL-6 were measured in the culture media by ELISA.

RESULTS: Both uPA and PAI-1 protein and mRNA expression were downregulated ($p<0.05$) in CTBs treated with >135 mg/dL glucose compared to basal (45 mg/dL). Anti-angiogenic factors (sENG, sFLT-1) and IL-6 were up-regulated, while the angiogenic factors (VEGF, PlGF) were down-regulated in the presence of >135 mg/dL glucose. The p38 MAPK phosphorylation and PPARγ expression were up-regulated ($p<0.05$) in hyperglycemia-exposed CTBs. SB203580 and rosiglitazone pretreatment attenuated glucose-induced down-regulation of uPA and up-regulation of p38 MAPK.

CONCLUSION: Exposure of CTBs to excess glucose inhibits the invasive profile of CTBs by decreasing the expression of uPA and PAI-1; by downregulating of VEGF and PlGF; and upregulating of sENG, sFLT-1, and IL-6. Attenuation of CTB dysfunction by SB203580 or rosiglitazone pretreatment suggests the involvement of stress signaling.

63 Macrosomia has roots in early placental development

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OBJECTIVE: Macrosomia (MACRO: ≥4000g) is associated with adverse neonatal outcomes and has been associated with modifiable risk factors such as weight gain and glycemic control. An early predictor of MACRO may identify patients for whom effective interventions can reduce their risk of macrosomia. We sought to determine if early placental size may be associated with the development of macrosomia.

STUDY DESIGN: 3D ultrasound volume sets were obtained at 11-14 weeks (N=578) and at 20-22 weeks (N=373) in a prospective cohort of singleton pregnancies. VOCAL (4DVIEW, GE) was used to calculate placental volume (PV). PV was normalized to gestational age to yield placental quotient (PQ). In addition, mean placental diameter (MPD) was the mean of 4 traced measurements of the maternal surface of the placenta taken every 45° degrees around the placental circumference. Potential confounders were included in multivariable regression models for the prediction of MACRO.

RESULTS: 7.6% (44/587) of our cohort had a MACRO infant. MACRO was associated with a higher median maternal age (33 vs 32y; P=0.09) and BMI (26.4. vs 24.6; P=0.01), a lower rate of nulliparity (4.6% vs 19.9%, P=0.01) and a higher rate of diabetes (6.8% vs 2.1%, P=0.08). Race was not significantly associated with MACRO (P=0.37). Both 1st and 2nd trimester placental measures were significantly associated with the development of MACRO (Table). These associations remained significant after adjusting for potential confounders. ROC analysis showed no significant difference in the predictive ability of the various adjusted models ($P>0.05$). Thus, 1st trimester placental measurements were similarly predictive of MACRO as 2nd trimester measures.

CONCLUSION: Early placental size is associated with the development of macrosomia even after adjusting for potential confounders. A non-invasive, point-of-care test that can identify those at greatest risk of MACRO may be useful in allowing for targeted counseling and interventions regarding diet, weight gain and diabetes screening that may impact pregnancy outcomes.

Placental Measure	Normal*	MACRO*	P-value	AUC alone	Adjusted AUC**
PV1 (cc)	65.8 (52.3-80.0)	74.5 (65.2-85.7)	0.006	0.6247	0.7235
PQ1	0.74 (0.60-0.90)	0.87 (0.75-0.98)	0.002	0.6395	0.7308
MPD1 (cm)	11.3 (11.1-11.4)	11.7 (11.3-12.2)	0.06	--	--
PV2 (cc)	237.9 (200.0-274.2)	265.8 (231.9-321.9)	0.005	0.662	0.7383
Volume/week (cc)	21.4 (16.8-26.0)	23.6 (20.1-30.8)	0.04	0.6438	0.7212
PQ2	1.7 (1.4-1.9)	1.9 (1.7-2.2)	0.002	0.6718	0.7462
MPD2 (cc)	15.5 (15.3-15.7)	16.7 (16.0-17.4)	0.0007	0.687	0.7719

*Descriptive data expressed as median (interquartile range); **1st trimester models adjusted for nulliparity, BMI, and DM; 2nd trimester models adjusted for nulliparity and DM.

64 In utero exposure to a maternal high fat diet Alters the epigenetic histone code in a murine model

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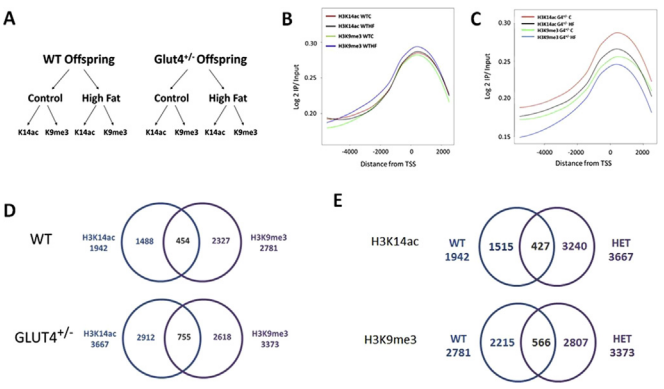
OBJECTIVE: Data from animal models show that in utero exposure to a maternal high fat diet (HFD) is deleterious to the health of the offspring, increasing the susceptibility to the adult onset of metabolic

syndrome. Epigenetic modifications may serve as a molecular memory of the in utero environment. Our objectives were to determine whether in utero exposure to a maternal HFD would be associated with an altered fetal histone code in a murine model, and to determine the metabolic pathways epigenetically reprogrammed with HFD exposure.

STUDY DESIGN: Both fetal and 5 week old Wild type (WT) and Glut4+/- (G4+/-) offspring of WT mothers exposed either to a control or a HF diet in utero were studied. Immunoblotting was utilized to determine hepatic changes in specific histone modifications (N=4/group). qPCR was utilized to determine gene specific expression in both the fetal and 5 week animals (N=4/ group). Chromatin immunoprecipitation (ChIP) followed by hybridization to promoter chip arrays (ChIP on chip, N=3/ group) was utilized to determine genomic localization of specific histone modifications in the fetal liver.

RESULTS: Immunoblotting revealed an increase in hepatic H3K14ac and H3K9me3 with HFD exposure in fetal WT (3.6-fold, p=0.002 and 5.7-fold, p=0.007) and G4+/- offspring (3.0-fold, p=0,002 and 4.6-fold, p=0.047), which persisted in the 5 week old offspring. Expression of the histone deacetylase, Sirt1, was decreased with HFD exposure in both WT and G4+/- 5 week offspring (0.45-fold, p<0.01 and 0.8-fold, p<0.01). Analysis of genes enriched for either H3K14ac or H3K9me3 in the fetal liver revealed differential enrichment within genes involved in lipid metabolism.

CONCLUSION: We conclude that HFD exposure in utero is associated with alterations in the fetal hepatic histone code in both WT and G4+/- offspring. We propose that the increase in hepatic H3K14ac and H3K9me3 in fetal life marks genes involved in lipid metabolism and may act as a molecular memory of the in utero exposure to a maternal HFD.



Chip on chip reveals a distinct profile for H3K14ac and H3K9me3 localization with maternal HFD exposure. (A) Livers from fetal WT or G4+/- animals exposed to either a control or a HFD in utero were used for ChIP using either H3K14ac or H3K9me3 antibodies. Localization of both H3K14ac and H3K9me3 in WT (B) or G4+/- (C) animals surrounding the TSS is calculated using Log 2 IP/IN. (D) In the WT animals, H3K14ac was differentially enriched in the promoters of 1942 genes with HFD exposure, and H3K9me3 in 2781 genes. There were 454 genes common to each group. In G4+/- animals, H3K14ac was differentially enriched in the promoters of 3667 genes, and H3K9me3 in 3373 genes. There were 755 genes common to each group. (E) In the WT offspring, 1942 are differentially enriched with HFD exposure, in the G4+/- offspring 3667 genes show differential enrichment. For H3K9me3, the WT offspring have 2781 and the G4+/- offspring have 3373 genes differentially enriched by virtue of HFD exposure.

65 ω -3 poly-unsaturated fatty acid supplementation in overweight and obese women: a pilot RCT to improve inflammation, insulin sensitivity and decrease fetal adiposity

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OBJECTIVE: We previously reported (ADA 2013) that ω-3 Poly-Unsaturated Fatty Acid (EPA/DHA, PUFA) supplementation decreases inflammation in placenta and adipose tissue of overweight/obese pregnant women (O/O). We now determine if ω-3 PUFA supplementation improved insulin sensitivity, inflammatory profile and decreases fetal adiposity.

STUDY DESIGN: We performed a double blind RCT in O/O randomly assigned ω-3 PUFA (800 mg DHA/1200 mg EPA, n=25) or placebo (wheat germ oil, n=24). Inflammatory markers, lipids, OGTT, body composition, diet and activity measures at 8-16 (V1) and 34-36 weeks (V2). Insulin sensitivity was measured using HOMA and ISogtt. Maternal and neonatal body composition was performed using anthropometry and air densitometry. Outcomes between groups were compared and changes from V1 to V2 were analyzed as the difference (Δ) between groups with adjustments for significant co-variables.

RESULTS: There were no significant differences in maternal age, parity, race, BMI, fat mass and gestational age at V1. Maternal weight (89.9±19.9 vs.79.9±10.6 kg, p=0.03) and lean mass (51.3±7.8 vs.46.9±4.8 kg, p=0.02) were higher in the ω-3 PUFA vs. placebo at V1. There were no significant differences in diet, activity, insulin sensitivity, plasma lipids, ω-3 PUFA and cytokine levels between groups at V1. The ω-3 PUFA concentrations (EPA 1.00±0.13 vs.0.2±0.02, p=0.001), (DHA 3.50±0.20 vs.2.40±0.10, p =0.001) and ω6/ω3 ratio (0.14±0.05 vs.0.07±0.01, p=0.003) were higher in the ω-3 PUFA vs. placebo group at V2. Differences (V2-V1) in plasma cytokines, insulin sensitivity and neonatal body composition are shown (Table). When adjusted for gestational age, gender, GDM, maternal lean and fat mass, there was no significant difference in neonatal body composition between groups.

CONCLUSION: Despite significant decreases in maternal plasma CRP, treatment of O/O with ω-3 PUFA did not modify maternal insulin sensitivity or neonatal body composition. (ARRA HD057236.)

	ω - 3 PUFA	Controls	p-value
Cytokines			
Adiponectin (ug/mL)	-1.43 ± 3.86	-1.78 ± 4.21	0.76
C-Reactive Protein (ng/mL)	-3205 ± 5128	-684 ± 6550	0.03
Leptin (ng/mL)	14.2 ± 22.9	5.3 ± 19.8	0.19
IL-6 (pg/mL)	7.4 ± 54.2	-0.3 ± 36.7	0.60
Insulin Sensitivity			
HOMA-IR	0.59 ± 1.52	0.46 ± 1.17	0.75
IS OGTT	-0.88 ± 2.83	-1.92 ± 3.27	0.25
Neonatal Anthropometrics			
EGA (weeks)	39.2 ± 1.6	38.7 ± 1.2	0.20
Gender (M/F)	15 / 10	8 / 16	0.06
Birth weight (g)	3278 ± 448	2935 ± 356	0.005
Lean mass (g)	2883 ± 310	2628 ± 250	0.003
Fat mass (g)	396 ± 160	307 ± 127	0.04
Body fat (%)	11.7 ± 3.8	10.1± 3.5	0.16
Pea Pod	(n= 20)	(n= 20)	
Lean mass (g)	2807 ± 328	2561 ± 229	0.009
Fat mass (g)	471 ± 139	448 ± 142	0.61
Body fat (%)	14.2 ± 3.4	14.7 ± 3.6	0.67