*Grand Ball Room A, New Orleans Hilton Riverside*

**9 HYPERGLYCEMIA DOWNREGULATES THE PKC-CPLA2 SIGNAL CASCADE IN EMBRYOS AND YOLK SACS OF DIABETIC RATS** E. REECE[1], S. JAMES[1], YINGKING WU[1], PATRYCJA KRAKOWIAK[1], MARIO CLEVES[1], STEPAN MELNYK[1], [1]University of Arkansas for Medical Sciences College of Medicine, Little Rock, AR

**OBJECTIVE:** In previous studies, we demonstrated that supplementation of pregnant diabetic rats with arachidonic acid (AA) reduced malformation rate, suggesting that AA deficiency is associated with diabetic embryopathy. In the present study, we compared the expression of protein kinase C (PKC) and phospholipase A2 (cPLA2), two enzymes that regulate phospholipid signaling and release of membrane AA, in conceptuses (embryos and yolk sacs) from normal and diabetic rats.

**STUDY DESIGN:** Diabetes was induced (hyperglycemia >250 mg/dL) in 8-week-old female rats by IV streptozotocin. A sustained-release insulin pellet was inserted and rats were mated after normalization of glucose levels (80-150 mg/dL). On gestational day (GD) 4, implants were withdrawn to induce hyperglycemia; rats were sacrificed on GD12 and conceptuses examined morphologically and stored at −80°C. Protein lysates (50 μg) were separated on 10% SDS-PAGE and protein expression was evaluated by Western blot using affinity-purified mouse monoclonal antibody for PKC and cPLA2. The resulting bands were quantified by densitometry among all groups.

**RESULTS:** Relative to the non-diabetic control rats, expression of PKC was significantly ($P<0.05$) decreased in normal conceptuses from the diabetic rats but even further significantly ($P<0.05$) decreased in malformed conceptuses. Similarly, a progressive and significant decrease in cPLA2 expression was observed in normal and malformed conceptuses from diabetic rats compared to the non-diabetic control rats.

**CONCLUSION:** Our current findings of the dysregulation of the PKC cascade (responsible for AA-dependent prostaglandin synthesis, cell proliferation, differentiation, and apoptosis) strongly support the key role of AA deficiency in the genesis of diabetic embryopathy.

**10 PLASMA ADIPONECTIN CONCENTRATIONS IN EARLY PREGNANCY AND SUBSEQUENT RISK OF GESTATIONAL DIABETES MELLITUS** MICHELLE WILLIAMS[1], CHUNFANG QIU[1], MARTIN MUY-RIVERA[1], SURAB VADACHKORIA[1], TARA SONG[1], DAVID LUTHY[2], [1]Swedish Medical Center, Center for Perinatal Studies, Seattle, WA [2]Swedish Medical Center, Obstetrics & Gynecology, Seattle, WA

**OBJECTIVE:** Low plasma adiponectin, a novel adipocyte-derived polypeptide, has been identified as a risk factor for insulin resistance and type 2 diabetes. Our objective was to determine the extent to which low maternal plasma adiponectin, measured in early pregnancy, is predictive of gestational diabetes mellitus (GDM), a condition of pregnancy that is biochemically and epidemiologically similar to type 2 diabetes.

**STUDY DESIGN:** We used a prospective, nested case-control study design to compare maternal plasma adiponectin concentrations in 41 patients who subsequently developed GDM with 70 controls. Study subjects were selected from a base population of 968 women who provided blood samples at an average gestational age of 13 weeks. Plasma apidonectin was determined using an enzyme-linked immunoassay. Logistic regression procedures were used to calculate odds ratios (ORs) and 95% confidence intervals (CI).

**RESULTS:** Adiponectin concentrations were statistically significantly lower in women who developed GDM as compared with controls (median concentrations: 4.4 vs. 8.1 μg/mL, $P<0.001$). Approximately 73% of women with GDM, as compared to 33% of controls, had adiponectin concentrations <6.4 μg/mL. After adjusting for confounding, women with adiponectin concentrations <6.4 μg/mL experienced a 4.9-fold increased risk of GDM, as compared with those with higher concentrations (OR = 4.9; 95% CI 1.9-12.4).

**CONCLUSION:** Our findings are consistent with other reports suggesting an association between hypoadiponectemia and subsequent risk of type 2 diabetes. Our findings extend the literature to include GDM. Studies designed to examine the effect of dietary, lifestyle, and pharmacological mediators of adiponectin concentrations in pregnant and non-pregnant subjects are warranted.

**11 ABNORMAL HOMOCYSTEINE METABOLISM AND GLUTATHIONE DEPLETION IN YOLK SACS AND EMBRYOS OF DIABETIC RATS** S. JAMES[1], YINGKING WU[1], STEPAN MELNYK[1], PATRYCJA KRAKOWIAK[1], MARIO CLEVES[1], E. REECE[1], [1]University of Arkansas for Medical Sciences College of Medicine, Little Rock, AR

**OBJECTIVE:** Exposure to maternal hyperglycemia induces both an increase in oxidative stress and a decrease in glutathione (GSH) in the developing embryo. We hypothesized that the resulting pro-oxidant microenvironment during vulnerable stages of organogenesis may contribute to hyperglycemia-induced malformations.

**STUDY DESIGN:** Diabetes was induced in 8-week-old female rats by IV streptozotocin injection. Glucose was monitored until diabetic levels of hyperglycemia (>250 mg/dL) were achieved. A sustained-release insulin pellet was then inserted subcutaneously. Rats were mated after normal and stable glucose levels (80-150 mg/dL) had been attained. On gestational day (GD) 4, the implants were withdrawn. Experimental rats were sacrificed on GD12 and embryos and yolk sacs were examined morphologically and stored at −80°C until analysis. Intracellular levels of oxidized (GSSG) and reduced glutathione (GSH) and the metabolic precursors, homocysteine (Hcy), methionine, cysteine (reduced), and cystine (oxidized) were analyzed by HPLC with electrochemical detection.

**RESULTS:** A 2-fold decrease in the redox ratio (GSH/GSSG) was observed in the malformed compared to normal embryos. In the yolk sac, a 3-fold decrease in GSH/GSSG ratio was observed in malformed embryos, indicating a significant increase in intracellular oxidative stress. A decrease in the glutathione precursors, methionine and cysteine, was associated with decreased GSH levels. The increase in Hcy and cysteine is consistent with hyperglycemia-induced inhibition of γ-glutamylcysteine synthetase, the rate-limiting enzyme for glutathione synthesis.

**CONCLUSION:** These data provide strong evidence that glutathione-mediated antioxidant defense mechanisms are compromised in embryos and yolk sacs subjected to maternal hyperglycemia during critical stages of organogenesis. The resulting pro-oxidant intracellular environment is considered critical in the genesis of diabetic embryopathy.

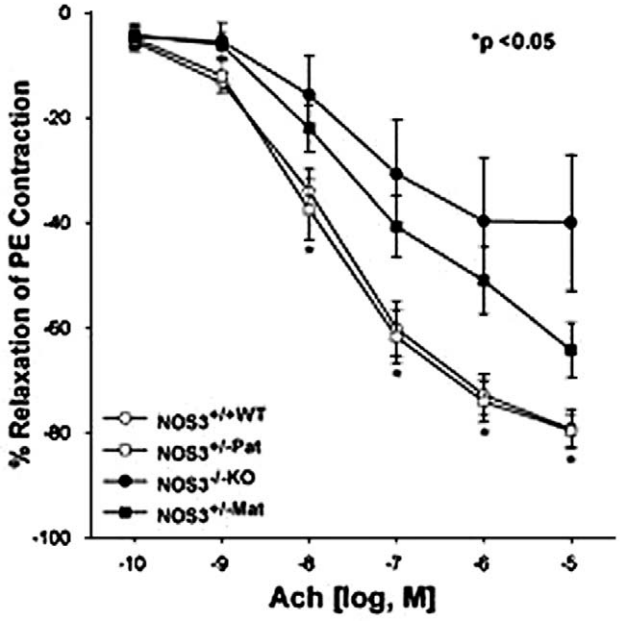
**12 FETAL ORIGIN OF DISEASE: THE CONTRIBUTION OF GENDER TO THE EFFECT OF UTERINE ENVIRONMENT AND GENETIC IMPRINTING ON VASCULAR REACTIVITY IN OFFSPRING OF TRANSGENIC MICE** JOSJE LANGENVELD[1], MONICA LONGO[2], YURI VEDERNIKOV[2], GARLAND ANDERSON[2], DIRK KIEBACK[1], ROBERT GARFIELD[2], GEORGE SAADE[2], [1]Maastricht University Medical Center, Dept. of Obstetrics & Gynecology, Maastricht, The Netherlands [2]University of Texas Medical Branch, Dept. of Obstetrics & Gynecology, Galveston, TX

**OBJECTIVE:** We have previously demonstrated that vascular responses in female mice heterozygous for disruption of endothelial nitric oxide synthase (NOS3) gene depend on the parental source of the abnormal gene. The objective of this study was to determine if gender modifies the contribution of the uterine environment and/or parental origin on vascular reactivity in later life.

**STUDY DESIGN:** Homozygous NOS3 knockout (C57BL/6J-$NOS3^{-/-KO}$) and wild-type mice were crossbred to produce 4 litter types: homozygous knockout ($NOS3^{-/-KO}$), maternally derived heterozygous ($NOS3^{+/-mat}$), paternally derived heterozygous ($NOS3^{+/-pat}$), and wild-type ($NOS3^{+/+WT}$) mice. Males from these litters were sacrificed at 7-8 weeks of age (n = 5-10/group) and 2 mm segments of carotid artery were mounted in a wire myograph for isometric tension recording. The effects of phenylephrine (PE, $10^{-10}$–$10^{-5}$ M), acetylcholine (ACh, $10^{-10}$–$10^{-5}$ M), and $Ca^{2+}$ (0.05-5 mM) in $Ca^{2+}$-free high-$K^+$ physiological solution were studied. Maximal effect, area under the concentration-response curve, and $EC_{50}$ (concentration producing 50% of the maximal effect) were calculated.

**RESULTS:** In the $NOS3^{+/-mat}$ and $NOS3^{-/-KO}$ mice, the vasorelaxant effect of ACh was significantly decreased (Figure), and the contractile responses to PE and $Ca^{2+}$ were significantly increased compared with $NOS3^{+/-pat}$ and $NOS3^{+/+}$ WT.

**CONCLUSION:** Despite one normal and one disrupted NOS3 allele in both maternally and paternally derived heterozygous pups, vascular function in later life is determined by the uterine environment and/or genetic imprinting. These data confirm our previous finding in females and demonstrate that fetal programming of vascular reactivity in later life is not gender-specific.




Acetylcholine