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CHARACTERIZING THE EARLY CRANIAL MESODERM: DEVELOPMENT OF THE ENDOTHELIUM.

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In classic models of craniofacial development, the cranial mesoderm was thought to play only a passive role, receiving signals from the migrating neural crest cells (NCC). Recent studies suggest that the cranial mesoderm can influence NCC identity and migration, suggesting a more active role for the mesoderm in craniofacial development. The goal of our study was 1) To identify genes specific to the cranial mesoderm that may influence NCC development, 2) To characterize their spatial and temporal expression, and 3) To evaluate the effect of identified genes on NCC development. Using an Affymetrix microarray, we have identified 184 genes expressed at a >3 fold difference in the cranial mesoderm when compared to a pooled endoderm/ectoderm sample. A large number of endothelial genes and genes involved in vascular development were identified by the screen, including *Vegf-C*, *Flk-1* and *Flt-1*, *Fli-1*, *Sox18*, *VE-cadherin*, *Esam1*, *Claudin 5* and *Igfbp4*, which suggests that development of the endothelium may play an important role in early craniofacial formation. *In situ* hybridization analysis demonstrates that the endothelial genes show diffuse punctate expression throughout the cranial mesoderm, and focus on endothelial gene *Igfbp4* demonstrates that *Igfbp4* is dynamically expressed in the developing branchial arches. Functional analysis of *Igfbp4* using bead implantation experiments demonstrates that *Igfbp4* is upregulated by FGF8 but is not influenced by SHH expression. Further work is underway to evaluate the effect of *Igfbp4* and other endothelial genes on NCC development using gene overexpression in *Pax3*-GFP transgenic mice.

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ADVERSE EFFECTS OF CAFFEINE ON EMBRYONIC CARDIAC FUNCTION DURING EARLY CARDIAC MORPHOGENESIS.

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Caffeine is a naturally occurring product that acts as a mild central nervous system stimulant. In humans the major sources of caffeine are coffee, tea, and soft drinks, as well as cocoa, chocolate, and certain medications. Caffeine is metabolized more slowly in pregnant women and due to the hydrophobic properties of caffeine it can cross the placenta and the brain-blood barrier. Studies in human and animal models have shown that caffeine exposure during pregnancy affects the perinatal cardiovascular system as well as central nervous system and can result in intrauterine growth retardation and stillbirth. Recent studies show that caffeine intake increases risk of first-trimester spontaneous abortion in human. However, the extent and the mechanism by which maternal caffeine intake influences embryonic cardiovascular function during early morphogenesis is not known. We hypothesized that caffeine ingestion during early pregnancy impairs embryonic cardiac function by delaying the onset of heart beat and alters the normal increase in heart rate (HR) resulting in growth delay and first-trimester spontaneous abortion. Eight to 12 week-old pregnant CD-1 mice and 81embryos were studied under an approved IACUC protocol. Caffeine was dissolve in distilled water and administered daily by gavage at a dose of 120mg/kg from gestational days 0.5 to 10.5. We monitored embryonic heart rate (HR) from gestational days 8.5 to 10.5 at 24 hour intervals using a 40MHz ultrasound biomicroscope. At gestational day 10.5, embryos were fixed and somite number and external morphology was assessed. This period of gestational includes the onset of heart beat of the primitive heart tube through the completion of heart looping. Onset of heart beat was significantly delayed in caffeine group at gestational day 8.5 (heart beat was detected in 41% of caffeine treated embryos versus 79% of sham treated embryos). HR increase was higher in caffeine group at gestational days 9.5 (127 ± 4 in caffeine vs. 112 ± 5 in sham, respectively, $p<0.05$) and 10.5 (150 ± 4 vs. 140 ± 4). At gestational day 10.5, caffeine treated mice had a significantly higher rate of embryo abortion (10% in caffeine vs. less than 2% in sham, $p<0.05$). Somite number was similar in both groups, however, body size, head size, and upper extremity length was significantly smaller in caffeine group. Thus, our results confirm that intrauterine caffeine exposure alters embryonic cardiac function (onset of heart beat, normal HR increase), embryo growth, and embryo survival during a critical period of early cardiovascular morphogenesis.

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CARDIOMYOPATHIC CHANGES IN OFFSPRING OF DIABETIC RATS.

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Background: Infants born to mothers with gestational diabetes are known to have organomegally and asymmetric septal hypertrophy. The purpose of this study is to evaluate the myocardial response in the offspring of severely diabetic rats and characterize the mitogen-activated protein kinase (MAPK) signaling pathways that may mediate these responses. **Hypothesis:** In a rat model of infant of diabetic mothers (IDM), cardiomyopathic changes develop in the offspring and that the MAPKs will regulate the cardiac responses. **Methods:** Pregnant rats were given either 50mg/ kg of streptozotocin or normal saline intravenously on day 7 of gestation (term=23 d). Maternal blood glucose levels were monitored. Animals were studied on days 18 (E18) and 21 (E21) of gestation, and postnatal days 1 (NB1), 5 (NB5) and 21 (NB21). Hearts were harvested and the ventricles weighed and then frozen in liquid nitrogen. MAPKs measured by Western blot included total and activated (phosphorylated) levels of c-jun-n terminal kinase 1 (JNK1 and pJNK1) and 2 (JNK2 and pJNK2) and extracellular signal-regulated kinase 1/2 (ERK1/2 and pERK1/2). NB1 and NB21 pups underwent echocardiographic study to evaluate left ventricular dimensions and function. **Results:** The mean heart to body weight ratio (HW/BW) was significantly elevated in the offspring of diabetic mothers when compared to controls due to a significant decline in BW (see Table). No differences were observed between controls and IDM offspring in the

	E 18	E 21	NB 1	NB 5	NB21
n	13	38	38	28	28
HW	94%	79%*	80%*	65%*	89%
BW	79%*	68%*	70%*	61%*	78%*
HW/BW	121%*	131%*	112%*	108%*	117%*

levels of ERK1/2, pERK1/2, JNK1, pJNK1 and pJNK2. Total JNK2 differed by age and treatment when analyzed by two-way ANOVA ($p<.05$). Echocardiographic analysis revealed a greater than two-fold increase in end-systolic LV volume and reduced LV ejection fraction in the NB1 IDM pups compared to controls although cardiac output was unchanged. LV dimensions and function were not different between IDM and control pups at NB21. **Conclusions:** With severe diabetes, IDM had diminished somatic growth that exceeds the decline in heart growth. A cardiomyopathy was present in the immediate newborn period that did not result in activation of the MAPK pathways and resolved by 21d postnatally.

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THYROID HORMONE INCREASES EAAT1 EXPRESSION IN RAT HEART.

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Background: Hyperthyroid induced cardiac hypertrophy is related to increased cardiac workload. These changes are associated with an upregulation of metabolic pathways associated with energy production. The malate/aspartate shuttle, necessary to transfer the reducing equivalents produced by glycolysis into the mitochondria, is increased 33% in hyperthyroid rats. Of the shuttles two inner membrane protein carriers, the aspartate-glutamate carrier is rate-limiting. The Excitatory Amino Acid Transporter, Type 1 (EAAT1) has recently been shown to function as a glutamate carrier in the malate/aspartate shuttle. We hypothesize that EAAT1 is upregulated by thyroid hormone. **Methods:** Adult Sprague-Dawley rats were infused with, tri-iodothyroxine (T3), propylthiouracil (PTU), or saline over a period of 8 days. Serum free T3 levels were measured. Rats were euthanized, hearts weighed, and tissue frozen. Northern Blot analysis was performed on total RNA using a unique 350 bp 32-P labeled EAAT1 ribonucleotide probe and normalized to 18S rRNA. A spectrophotometric assay of the malate/aspartate with glutamate and lactate as substrates was performed on isolated mitochondria. Results are displayed as oxidation rate/min/mg mitochondrial protein. Protein lysates from mitochondria were used for immunoblot analysis with human anti-EAAT1 IgG. **Results:** EAAT1 steady-state mRNA levels were increased in the T3-treated rats compared to controls ($0.031+/-0.005$ vs. $0.011+/-0.002$; $P<0.05$), and decreased in PTU-treated rats vs. controls ($0.0011+/-0.0002$ vs. $0.0015+/-0.0001$; $P<0.05$). EAAT1 mitochondrial protein levels were increased in T3-treated rats vs. controls ($8.9+/-0.4$ vs. $5.9+/-0.6$; $P<0.005$). EAAT1 protein levels were below detection in PTU-treated rats. Malate/aspartate shuttle activity was unchanged by PTU infusion. **Conclusions:** Hyperthyroidism in rats is related to an increase in expression of both EAAT1 mRNA and protein in cardiomyocytes. This 49% increase in the rate-limiting aspartate-glutamate carrier of the malate/aspartate shuttle correlates with the observed increase in shuttle activity. The upregulation of EAAT1 by thyroid hormone may facilitate the cardiomyocyte response to hyperthyroidism and the associated increased metabolic demand placed upon the cell.

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AN EDUCATIONAL REMEDIATION PROGRAM BENEFITS CHILDREN WITH SICKLE CELL DISEASE AND CEREBRAL INFARCTS.

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The overall goal of this project was to determine the feasibility of an educational remediation program for children with sickle cell disease (SCD) and cerebral infarcts. Approximately 30% of children with SCD have cerebral infarcts before eighteen years of age, at least 60% of this group have been retained a grade in school, and 79% will have a cognitive deficit. We performed a prospective randomized pilot trial for children with SCD, cerebral infarcts and memory deficits. Participants were randomly allocated to either a control or intervention group to test the following hypothesis: Targeted memory strategy remediation will have a greater improvement in (1) memory skills and (2) academic achievement of children with SCD, cerebral infarcts, and memory deficits than in the same group of children who receive general tutoring. Both the control and intervention groups received general tutoring for four semesters; additionally, the intervention group received specific remediation strategies to improve their memory skills. In the first year, tutors met with students for one hour per week; this was increased to two-one hour sessions per week during the second year. Parents were asked to spend at least the same amount of time with their children each week. The primary outcome measure was assessment of memory performance before/after completion of the intervention. Secondary outcomes were Wechsler Individual Achievement Tests (reading, math, spelling). Nine of eleven children completed the two-year program. Children in the intervention group significantly improved their abilities in two measures of memory: delayed cued memory (19 point improvement, $p=.01$) and working memory (31 point improvement (2SD) in the digits backward measure, $p=.04$). Both groups had a range of improvement (1–26 points) in academic achievement tests, but these gains did not reach statistical significance. Unfortunately, most parents did not or could not follow through with tutorials or practicing memory drills at home. In conclusion, we have provided preliminary results that suggest memory remediation may be a feasible method to improve memory skills for children with SCD and cerebral infarcts and provide evidence for further studies in this area of investigation.

MWSPR PLENARY SESSION II

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RELATIONSHIP BETWEEN DAYTIME OXYGEN DESATURATION AND SLEEP RELATED BREATHING DISORDER IN CHILDREN WITH SICKLE CELL ANEMIA.

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Recent studies strongly suggest that nocturnal oxygen desaturation in children with sickle cell anemia is associated with an increase incidence of pain and stroke. However, no relationship has been established between daytime oxygen desaturation and sleep disturbance. To determine the relationship between daytime oxygen desaturation and sleep related breathing disorder in children with sickle cell anemia we evaluated 17 patients with HbSS disease referred to the sleep laboratory at St. Louis Children's Hospital. All patients were referred for daytime oxygen desaturation defined as less than 94% on room air by pulse oximetry. Each patient underwent an overnight recorded polysomnogram with sleep guidelines and parameters following the general consensus statement and standards set by the American Thoracic Society. Results showed 82% of the patients had nocturnal hypoxemia with overnight mean oxygen saturation less than 94% with values ranging from 75–95% and a mean oxygen saturation of 89%. 18% of the patients had an oxygen saturation less than 85% overnight, and 65% of the patients had a formal recommendation of nocturnal supplemental oxygen use after the polysomnogram. 53% of the patients had a diagnosis of Obstructive Sleep Apnea Syndrome (OSAS) with an Apnea Index range of 0.1–7.5 and a Respiratory Disturbance Index range of 4–41.4. Our preliminary results suggest that patients with sickle cell anemia have an increased risk of sleep related breathing disorder including OSAS and nocturnal hypoxemia. Patients with low daytime oxygen desaturation are at risk for sleep related breathing disorders. Further prospective evaluations are underway to validate these findings and to elucidate the etiology of sleep disturbances in this vulnerable population.

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