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### REDUCED INTENSITY CONDITIONING THERAPY USING CAMPATH-1H IS SUCCESSFUL FOR STEM CELL TRANSPLANTATION IN NON-MALIGNANT DISORDERS.

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Stem cell transplantation (SCT), indicated for many non-malignant disorders, is limited by donor availability, graft rejection (GR), toxicities of conditioning, morbidity and mortality (TRM), and graft versus host disease (GVHD). To overcome these barriers, we tested a novel conditioning for SCT. It was designed to support engraftment by deleting host immune reactive lymphocytes and macrophages. Campath-1H (anti-CD52,mab) was given on days -21, -20, and -19 (total dose 48 mg), fludarabine (day -8 to -4) (total 150 mg/m<sup>2</sup>) and melphalan on day -3 (140 mg/m<sup>2</sup>). Stem cell sources were related/unrelated bone marrow (BM) (8), peripheral blood (PB) (5) and umbilical cord blood (UCB) (3). GVHD prophylaxis was cyclosporine (tapered after 3 months), methylprednisone (tapered after day +28) and methotrexate on days +1, +3, and +6 (except in UCBT). End points studied were engraftment and TRM. Sixteen patients (1.5-40 yrs) with aplastic anemia (5), Hurler's (2), sickle cell anemia, XLAAD, histiocytosis (3), thalassemia, adrenoleukodystrophy, Evan's syndrome and dyserythropoietic anemia were transplanted. Median follow-up was 219 days (66-845). The regimen was tolerated well. All patients that survived >1 month engrafted. Neutrophils (ANC >500/dL) engrafted at 12.5 days; platelets (>50,000/dL) at 21 days (median). Skin GVHD developed in 3 patients and resolved early. Eight patients are off; 4 are tapering immune suppression. All survivors have either stable disease or are cured. One survivor had a normal pregnancy. Two patients died prior to engraftment from previously acquired Pseudomonas infection. Two died of CMV disease and intracranial hemorrhage/refractory thrombocytopenia respectively after engraftment. Other complications were bacterial and viral infections occurring within 100 days. Profound lymphopenia was present 1 month. NK cells recovered by 3 months. CD8+ cells by 6 months, CD4+ and B cells 6-9 months after transplant. Immunoglobulin (Ig M and A) levels reflected B cell numbers; Ig A recovered later than IgM. In summary, successful engraftment despite varied stem cell sources was achieved without significant GVHD using this regimen. Lymphopenia resulted in a significant infection risk early post transplant, requiring close surveillance and intervention. Thus, this transplant regimen is well tolerated and may preserve fertility, making it a promising alternative to conventional SCT.

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### EARLY GESTATION DEXAMETHASONE EXPOSURE ALTERS CALCIUM TRANSIENTS IN CORONARY VASCULAR SMOOTH MUSCLE CELLS IN NEWBORN LAMBS.

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**Background:** Fetal programming of adult diseases, including coronary artery disease, may be a consequence of fetal exposure to increased levels of maternally derived glucocorticoids. In an established ovine model, early gestation glucocorticoid exposure alters postnatal coronary artery vascular reactivity. This programming effect may be related to modifications in intracellular calcium regulatory responses. **Hypothesis:** To determine if early gestational glucocorticoid exposure alters agonist-induced cytosolic calcium concentrations in coronary artery vascular smooth muscle (VSM). **Methods:** Dexamethasone (dex, 0.28 mg/kg/d IV for 48 h) was administered to pregnant ewes at 27-28 days gestation (term 145 d). Ewes were allowed to deliver and the offspring and control lambs studied at a postnatal age of 12 ± 3 d (n=3 for each group). Intracellular calcium responses of primary cultured coronary and carotid VSM cells to a variety of agonists were determined in Fura-2 (a fluorescent Ca<sup>2+</sup> indicator) loaded cells using a calcium imaging system (n = 8-40 cells for each condition). **Results:** Baseline [Ca<sup>2+</sup>]<sub>i</sub> was similar in control and dex-exposed coronary VSM. Dex-exposed coronary VSM displayed decreased peak calcium responses to angiotensin II (1 μM) and bradykinin (1 μM) compared to controls; peak responses to endothelin-1 (1 μM) and the thromboxane agonist U46619 (10 μM) were similar in both groups. KCl (90mM) caused an increase in [Ca<sup>2+</sup>]<sub>i</sub> (322 ± 35 nM) in control VSM but had no effect on dex VSM. The [Ca<sup>2+</sup>]<sub>i</sub> response to U46619 in control but not dex VSM was attenuated by the calcium channel blocker nifedipine (10 μM). Conversely, KCl increased [Ca<sup>2+</sup>]<sub>i</sub> in dex-exposed carotid VSM, and nifedipine decreased the calcium response to U46619. Dex also enhanced changes in [Ca<sup>2+</sup>]<sub>i</sub> in response to angiotensin II and U46619 in carotid VSM. **Conclusion:** Early gestation glucocorticoids program altered regulation of VSM [Ca<sup>2+</sup>]<sub>i</sub> in a vessel-specific manner. In particular, dex diminished apparent function of voltage-gated calcium channels in coronary but not carotid VSM. These observations suggest a mechanism whereby an altered in-utero environment may predispose to coronary artery dysfunction later in life.

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### EARLY GESTATION DEXAMETHASONE EXPOSURE ALTERS CORONARY ARTERY REACTIVITY IN NEWBORN LAMBS.

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**BACKGROUND:** Exposure of the ovine fetus to glucocorticoids early in gestation programs postnatal elevation of blood pressure and enhanced coronary artery reactivity to second messenger-dependent vasoconstrictors. It is unknown whether the coronary alterations are a direct consequence of the corticosteroid exposure or related to the hypertension that evolves over the first 4 months of life. **HYPOTHESIS:** Early gestation glucocorticoid exposure enhances newborn lamb coronary artery vascular reactivity in the absence of systemic hypertension. **METHODS:** Dexamethasone (dex, 0.28 mg/kg/day iv for 48 hours) was administered to pregnant ewes at 27-28 days gestation (term being 145 days). The ewes were allowed delivery, and offspring were studied at a postnatal age of 8 ± 2 days (N = 6). Non-dex exposed age-matched control lambs were used for all comparisons (N = 6). Vascular catheters were placed 48 h prior to recording blood pressures. The contractile responses of circumflex coronary, mesenteric and femoral artery rings were then measured by wire myography. **RESULTS:** Exposure to dex was not associated with alterations in mean arterial blood pressure or heart rate at 8 days of life. Coronary vessels from dexamethasone-exposed sheep exhibited enhanced vasoconstriction to endothelin-1 and acetylcholine (both P < 0.05). There was no difference in maximal response of the coronary arteries to potassium chloride or angiotensin II. Dex exposure was associated with attenuation in vasodilatation to adenosine, but not sodium nitroprusside or forskolin. No differences in contractile response were detected between dex-exposed and control mesenteric or femoral arteries. **CONCLUSION:** Early gestation glucocorticoid exposure selectively programs postnatal alterations in coronary artery vascular reactivity prior to the development of hypertension. These findings suggest coronary artery dysfunction is a primary programming phenomenon and not secondary to alterations in blood pressure. These coronary vascular alterations may provide a mechanistic link between an adverse intrauterine environment and later coronary artery dysfunction.

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### GESTATIONAL AGE AFFECTS ACTIVATION OF THE MITOGEN-ACTIVATED PROTEIN KINASES AND AKT IN THE CHRONICALLY ANEMIC FETAL SHEEP HEART

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**Background:** The postnatal heart responds to biomechanical stress by myocyte hypertrophy, whereas the fetal heart may additionally undergo hyperplasia. Before 100 d gestational age (GA), nearly 100% of fetal sheep cardiomyocytes are mitotically active, which decreases to about 20% near term. The mitogen-activated protein kinases (MAPKs) and Akt are hypertrophic signaling pathways in adult hearts. The activities of these pathways are not well characterized in the loaded fetal heart. **Objective:** To test the hypothesis that activation of myocardial p38, c-jun-N-terminal kinase (JNK), extracellular signal-regulated kinase 1/2 (ERK 1/2) and Akt by increased cardiac load resulting from chronic anemia is developmentally regulated in early versus late GA sheep. **Methods:** Anemia was created in fetal sheep at 98 or 134 d GA (term 145 d) by daily isovolemic hemorrhage (20-30 ml or 60-100 ml, respectively) for 7 d (n = 7 for both ages). Age-matched, non-bled twins served as controls. Right (RV) and left ventricular (LV) MAPK and Akt protein levels were determined by Western blot. Data are given as mean ± SE. **Results:** In 98 d anemic fetuses, hemoglobin (9.4±0.3 g/dL) and arterial oxygen content (7.8±0.4 to 3.3±0.3 mL O<sub>2</sub>/dL) decreased significantly from days 1 to 8 of the study, while total heart weight normalized to body weight was significantly increased (6.69±0.3 vs 5.69±0.2 g/kg) compared to controls (p<0.05). RV and LV total and active protein levels of JNK, ERK 1/2 and Akt were similar between 98 d anemic and control fetuses, as were total levels of p38. Compared to the 134 d anemic fetuses, the 98 d anemic fetuses showed significantly greater RV and LV total and active protein levels of ERK and Akt (p<0.05). Total levels of LV p38 and RV and LV JNK were increased in 98 d versus 134 d anemic fetuses although active levels of RV and LV JNK and total levels of RV JNK were unchanged. **Conclusions:** In contrast to the late GA fetal heart, activity of MAPK pathways in early GA fetal myocardium is not altered by chronic anemia, indicating MAPKs are not essential for adaptation to an increased load. Volume loading of the fetal sheep heart leads to developmentally regulated cell signaling profiles - possibly related to the high percentage of mitotically capable myocytes in the immature fetal heart.

## MWSPR PLENARY SESSION III

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### FOOD AS A RISK FACTOR FOR GASTROESOPHAGEAL REFLUX SYMPTOMS IN ADOLESCENTS.

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Gastroesophageal Reflux (GER) is a common GI disorder. We reported a prevalence of 38% of esophageal GER symptoms among adolescents<sup>1</sup> and found cigarette smoking, alcohol and non-steroidal anti-inflammatory drugs (NSAIDs) were risk factors. Now we are analyzing if certain foods and drinks are risk factors for GER symptoms in the same age group. **Aim:** To find out the association between GER symptoms and the following as risk or protective factors: spicy foods, citrus fruit juices, 12 caffeinated and 15 non-caffeinated beverages, obesity, NSAIDs, alcohol, smoking and chewing gum. **Methods:** A cross sectional survey was done among 14-18 year old students at a high school. The survey instrument contained questions on esophageal (heartburn, regurgitation and dysphagia), respiratory symptoms (cough and shortness of breath) over the past year measured by symptom frequencies on a 6-point scale<sup>2</sup> and questions on the proposed risk factors. The data were entered into a MS Access Database and analyzed using SPSS. **Results:** Drinking coffee or tea, caffeine containing carbonated drinks (Barq's root bear, Dr. Pepper, Diet Dr. Pepper) and caffeine-free carbonated drinks (Sierra mist, Barq's diet root bear, A & W root bear, IBC root bear, Mug root bear, 7-Up, ginger ale, caffeine-free Coke, and Fanta) were found to be risk factors. Spearman's rho was between 0.10 to 0.30 and p value less than 0.05. Eating spicy foods, drinking citrus fruit juices or chocolate drinks were not risk factors. Subjects with greater BMI tended to have more frequent GER symptoms (rho=0.11, p=0.016). As we showed earlier<sup>2</sup>, alcohol, NSAID use and cigarette smoking were found to be risk factors (Odds ratio: NSAIDs = 1.38, cigarettes = 1.76, alcohol = 1.35, p<0.05). **Conclusion:** Certain carbonated caffeine containing and caffeine free drinks were found to be risk factors for GER symptoms. Coffee drinking had a higher risk than tea for GER symptoms. Contrary to our previous study<sup>2</sup>, increasing BMI was a risk factor. Use of NSAID, alcohol and cigarette smoking were risk factors for GER symptoms. Chewing gum was not found to be protective for GER symptoms.

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### ANALYSIS OF WEIGHT LOSS OF PEDIATRIC PATIENTS INVOLVED IN A HEALTHY LIFESTYLES CLINIC (HLC).

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Pediatric obesity has become pandemic accompanied by various health risks. Structured, non-uniformed weight (wt) loss programs are being established at pediatric facilities across the country. We have organized a program utilizing a multidisciplinary team of nutritionists, physicians, and PharmD's called our HLC. **Purpose:** To assess the outcome data as to the efficacy of HLC involving obese pediatric pts. **Methods:** A retrospective chart review of obese pediatric pts enrolled in our university-based HLC. Initial aulographic data were compared with last available data. **Results:** Data were reviewed on 218 obese pts (81M/137F). 137 pts were analyzed who had >1 visit to HLC (47M/90F; AA=76.6%, C=19.7%, H=1.5%, O=2.2%; age 11.4±3.2yrs). F/u visits

Ethnicity	Cauc. (n=27)	AA (n=105)	Other (n=5)	All (n=137)
Wt A (kg)	-2.0±6.3	1.7±6.7	0.1±5.1	0.9±6.7
Wt A - MTF	-5.1±9.0	0.4±5.6	-1.4±3.4	-0.5±6.3
Wt A - MTF	-0.4±2.1	2.1±6.8	2.4±8.1	1.5±6.0
Δ BMI (kg/m <sup>2</sup> )	-2.3±5.9	1.1±3.2	0.0±2.4	-0.3±3.9
Sex	(n=11)	(n=10)	(n=35)	(n=70)
Wt A (kg)	-3.9±8.8	-0.7±3.5	-1.1±10.0	0.6±3.9
Wt A - MTF	-7.0±10.7	-1.4±2.8	1.5±7.4	-0.2±4.4
Wt A - MTF	1.2±1.6	-1.0±1.9	4.2±11.0	1.1±3.2
Δ BMI (kg/m <sup>2</sup> )	-4.1±8.5	-0.8±1.5	0.5±4.3	0.0±2.4

numbered 2-10 (3.4±1.7; median=3) over 0.5-30 mos (6.3±5.6 mos; median=11.7). 64 pts [24M/40F; AA=81.3%, C=14.1%, H=1.5% (n=1), O=3.1% (n=2) received metformin (MTF). Insurance status: Private=30.7%, Medicaid=54.0%, 15.3% not documented.

Only some pt groups had statistically significant decrease in wt, but of questionable clinical significance: C vs Total Group (p=0.04); C♂ vs AA♂ (p=0.02). For pts w/o Rx w/MTF, C had significant wt loss vs Total Group (p=0.03) and vs AA (p=0.02). C♀ had significant wt loss vs C♂ (p=0.04) and vs All females (p=0.03) and vs AA♀ (p=0.01). Addition of MTF, led to further wt loss for C pts of both genders but p=NS due to wide SD. BMI Δ was not significant for any group. **Conclusion:** Our HLC had limited success.