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### KINETICS OF PRIMARY EPSTEIN-BARR VIRUS INFECTION AFTER PEDIATRIC HEART TRANSPLANTATION

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**Background:** Primary Epstein-Barr virus (EBV) infection frequently occurs early after transplantation (Tx) in seronegative pediatric heart Tx recipients who receive organs from seropositive donors and is the key risk factor for the development of post-transplant lymphoproliferative disorder (PTLD). The routine use of post-Tx EBV-PCR monitoring offers the opportunity to study the time course of primary EBV infection and the influence of differing immunosuppressive regimens. **Methods:** We studied 15 consecutive seronegative recipients who received a primary heart Tx from a seropositive donor. All were managed with tacrolimus-based immunosuppression with 9/15 also receiving 5 days of induction with rabbit antithymocyte globulin (Thymoglobulin). Surveillance was by whole blood real-time EBV-PCR approximately monthly for the first 6 months, then every 2 months for 6 months, then approximately quarterly thereafter. **Results:** Mean age at transplant was 5.4 years, and mean donor age was 8 years. Positive donor EBV serostatus prior to any donor transfusion was confirmed in 6 cases. One patient developed PTLD. A total of 270 PCR measurements were made (average 18/pt). 12/15 experienced primary EBV infection with first positive PCR 24-307 days post-Tx; median 38 days (Thymoglobulin pts 33d *vs*. non-Thymoglobulin pts 93d). Of 12 patients who developed detectable viral loads, 7 (58%) did so within 6 weeks of Tx. *Peak* EBV viral load ranged from 2,600 to 380,000 copies/ml; median 28,500 copies/ml (Thymoglobulin pts 26,000 *vs*. non-Thymoglobulin pts 47,000). Time to *peak* viral load was 32-1023 days, median 177 (Thymoglobulin pts 121d *vs*. non-Thymoglobulin pts 239d). Of the 3 patients who never developed a measurable viral load, none had pre-transfusion confirmation of donor seropositive status. Seronegative recipients with seronegative donors have not demonstrated early (first six weeks) viral loads, and screening of filtered blood products are also EBV-PCR negative suggesting that the loads observed in this study were not due to blood product use during cardiopulmonary bypass. **Conclusions:** EBV seronegative pediatric Tx recipients receiving hearts from seropositive donors experience very early EBV infection with evidence of viremia often within 4-6 weeks of Tx. Induction therapy may be associated with earlier viremia. EBV-PCR screening more frequently than monthly maybe indicated early after Tx in this population if any form of experimental / therapeutic intervention is planned at the time of primary EBV infection.

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### CHANGES IN TNFR1 LEVELS IN THE FIRST WEEK POST-MYELOABLATIVE ALLOGENEIC BMT CORRELATE WITH GVHD AND TRM IN PEDIATRIC PTS.

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Tumor necrosis factor (TNF) plays a crucial role in the pathogenesis of graft-vs-host disease (GVHD), the major cause of treatment-related mortality (TRM) after allogeneic bone marrow transplantation (BMT). We tested the hypothesis that early rises in TNF (on day 7 following BMT) predict the development of significant GVHD and TRM. Ratios of soluble TNF receptor 1 (TNFR1) levels (day 7 level/pre-BMT level) was used as a surrogate marker for TNF in 440 myeloablative allogeneic BMT pts with a median age of 42y (range 0-65y). There were 269 (61%) related donors pts and 171 (39%) unrelated donors pts. 82 pts (19%) of this cohort were children 17y and younger of whom 38 were related donor pts (46%), 38 unrelated donor pts (46%) and 6 cord blood pts (8%). The median day of onset of GVHD 2-4 was 27d. For the entire cohort, the mean day 7 TNFR1 ratio correlated with severity of GVHD ($p<0.001$). When analyzed for pediatric pts only, higher TNFR1 ratio continued to correlate with increasing severity of GVHD ($p = 0.01$). The highest quartile of day 7 TNFR1 ratios also strongly correlated with likelihood of GVHD 2-4, 1yTRM, and 1y survival in the complete cohort and children only.

| TNFR1 ratio | GVHD 2-4 | 1y TRM | 1y Survival |
|---|---|---|---|
| | | All patients (n=440) | |
| TNFR1 ≤ 2.5 (n=330, 75%) | 25% | 16% | 64% |
| TNFR1 > 2.5 (n=110, 25%) | 46% | 39% | 50% |
| | p<0.001 | p<0.001 | P<0.008 |
| | | Children (n=82) | |
| TNFR1 ≤ 2.5 (n=63, 76%) | 33% | 6% | 77% |
| TNFR1 > 2.5 (n=19, 24%) | 58% | 26% | 53% |
| | p=0.02 | p=0.07 | p<0.001 |

We conclude that the magnitude of rise in early post-transplant TNFR1 ratios correlates with the subsequent severity of GVHD, TRM and survival. These informative changes are detectable often two to three weeks in advance of clinical manifestations of GVHD and may therefore provide the basis for development of a predictive laboratory test.

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### LARGER LESS FREQUENT MEALS DECREASE HUNGER IN THE PEDIATRIC OBESE POPULATION

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Childhood obesity is increasing at an alarming rate. Studies of the mechanisms of abnormal weight gain are rare in the pediatric population. Abnormal weight gain has been associated with impaired control of appetite. Studies in lean adults have demonstrated that frequent small meals may increase satiety and decrease hunger. **Purpose:** To examine the effect of frequent feedings of low caloric density versus less frequent feedings of high caloric density on hunger and satiety in lean and obese prepubertal children. We hypothesized that frequent low caloric density feedings should increase satiety and lead to decreased hunger. **Methods:** Fifteen lean and eight obese children (age 6-10) were admitted fasting to the General Clinical Research Center (GCRC) on two separate days. On each of the two study visits children were randomly assigned to receive one of two meal patterns: either 3 meals or 3 meals and 2 snacks. The total caloric intake as well as carbohydrate, protein, and fat composition on each day were identical. Each day, two hours following the last meal, subjects were offered a dessert (ice cream) and asked to consume as much as they would like (up to a predetermined upper limit). Hunger related questionnaires and amount of ice cream consumed were recorded as outcome measures of satiety. Paired t tests were used to evaluate the difference between ice cream consumption as affected by feeding patterns. Each lean and obese subject served as their own control. Data from parental questionnaires were also collected. These questionnaires assessed if restrictive feeding practices in parents were correlated to ice cream consumption in the children. **Results:** Preliminary results indicate that obese children consumed significantly less ice cream on the day when larger, less frequent feedings were administered ($p<0.05$). No difference was found between ice cream consumption and meal patterns in lean subjects. In lean subjects, a correlation ($r=0.523$) at a p value of 0.05 was observed between greater scores of parental restriction of food and ice cream consumption. Data from hunger questionnaires in all subjects did not reveal a significant relationship between the hunger-rating instrument used and amount of ice cream consumed. **Conclusions:** Our preliminary data, as opposed to our hypothesis, indicate that obese children may experience greater satiety when offered larger meals without snacks. No relationship with meal patterns and ice cream consumption was found in the lean pediatric population. In the lean group, greater parental restriction of "forbidden" food correlated with greater consumption of that food when the restriction was absent

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### BOTH INSULIN SENSITIVITY AND DISPOSITION INDEX FALL DURING NORMAL FEMALE PUBERTY WITHOUT A CHANGE IN GLUCOSE TOLERANCE.

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**Purpose of Study:** There is controversy about whether the normal pubertal decrease in insulin sensitivity (Si) is compensated by an appropriate increase in $\beta$-cell function (BCF), as reflected in their product (disposition index, DI), particularly in African-Americans (AA). It has been postulated that this is because of the predisposition of AA to diabetes. However, the methodological intensity of methods to measure Si has previously precluded simultaneous assessment of glucose tolerance by an oral glucose tolerance test (OGTT) in children. We have circumvented this problem by applying a recently developed oral minimal model (OMM) to frequently sampled OGTT (fsOGTT) data. We assessed glucose-insulin dynamics across puberty in healthy females by the fsOGTT. **Methods used:** Healthy female volunteers underwent an fsOGTT, with assays of insulin, C-peptide, and glucose, as well as baseline plasma insulin-like growth factor-I (IGF-I). Those with normal OGTT (6 yr to 50 yr, n=52) were 42% AA, 42% Caucasian (C) and were grouped by pubertal stage (prepubertal, early pubertal, postmenarcheal < 18 years, and adult). Body mass index percentile was similar among stages, averaging 63rd-85th percentile. Si, BCF, and DI were derived from the OMM; other indices of Si and BCF were also computed. Statistical analysis was by analysis of variance and covariance, and Kruskall-Wallis tests, with post-hoc Dunn's tests. **Summary of results:** Si by OMM fell from 22.8 ± 3.1 (SEM) dL $kg^{-1}$m/mU/mL $10^{-4}$ prepubertally to 8.3 ± 2.2 post-menarcheally ($p\leq0.01$) and then recovered to 12.0 ± 4.4 in adults. Several other indices showed these changes, but others were less sensitive (e.g., HOMA-IS). Neither BCF (by OMM or insulin response to glucose) nor glucose changed significantly with stage. Consequently, DI by OMM followed the Si pattern of transient pubertal decrease ($p<0.02$). The IGF-I pattern was reciprocal ($p<0.001$).**Conclusions:** We confirm that DI declines in parallel to Si during normal puberty. Additionally, we show in an ethnically diverse population that this occurs in the absence of glucose intolerance and does not persist into adulthood. This implies that insulin-independent glucose metabolism increases during normal puberty. This coincides with the pubertal peak in IGF-I levels. We suggest that the fall in Si induced by the known pubertal rise in GH secretion is countered by the insulin-sparing effect of the concomitant rise in IGF-I.

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### EFFECT OF ERYTHROPOIETIN ON FLUID MOVEMENT ACROSS THE RESPIRATORY EPITHELIUM.

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Erythropoietin has a wide range of nonhematopoietic effects in the body, including the regulation of certain enzymes in the lung. Clinically, its administration has been associated with a decrease in the number of days in oxygen for sick premature infants. If erythropoietin has a favorable effect on respiratory illness, one mechanism could be a reduction in pulmonary edema. In previous studies, we demonstrated that erythropoietin increases cellular uptake of sodium, a necessary first step in ion transport-linked fluid clearance (*Pediatr Res* 58:817A, 2005). To further characterize the effect on ion transport, we studied the effect of erythropoietin on the Na-K-ATPase, an enzyme important in sodium translocation. To accomplish this goal, we treated A549 cells, an immortal adenocarcinoma cell line with features of Type II cells, with erythropoietin and measured the uptake of $^{86}Rb^{+}$ (a mimic of $K^{+}$) in the presence and absence of ouabain, an inhibitor of Na-K-ATPase. We found that after 24h, erythropoietin (35 U/ml) increased ouabain-sensitive Rb uptake on average by 25% (n=8, control: 1.6 ± 0.6 vs erythropoietin: 2.0 ± 0.8 nmol Rb/million cells/min, $p<0.05$), indicating an increase in Na-K-ATPase activity. To confirm this finding, we disrupted the cell membrane and assessed Na-K-ATPase activity by an alternative method in which we measured the release of inorganic phosphate in the presence and absence of ouabain. Similar to our results in intact cells, erythropoietin (24h, 35 U/ml) increased the activity of the Na-K-ATPase (n=7, control: 0.9 ± 0.4 vs erythropoietin: 2.1 ± 1.3 mg phosphate/mg protein, $p<0.05$). In additional studies, we grew A549 cells on a semipermeable membrane and measured fluid movement across the monolayer by measuring the change in protein concentration in the supernatant as a reflection of fluid loss. After 24 h, erythropoietin increased by nearly 25% the volume of fluid loss from the supernatant (n=6, control: 132 ± 26 vs erythropoietin: 164 ± 34 *u*L/24 h, $p<0.05$). We then added amiloride (1mM), an inhibitor of Na channels important in the transepithelial movement of liquid, to monolayers treated with erythropoietin and found that fluid loss from the supernatant decreased to that of control cells (n=6, control: 126 ± 26 *u*L/24 h). Our findings show that erythropoietin increases Na-K-ATPase activity and transepithelial liquid movement *in vitro*. We speculate that if erythropoietin were to increase sodium transport and Na-K-ATPase activity *in vivo*, then lung water balance and respiratory difficulty might improve as well.

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### EFFECTS OF MATERNAL GESTATIONAL DIABETES ON VASCULAR REACTIVITY IN ADULT RAT FEMALE OFFSPRING.

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**Background:** Exposure to diabetes *in utero* produces an adverse environment for the fetus and is a significant risk factor for the development of metabolic syndrome (obesity, glucose intolerance and hypertension). Previous studies have found that offspring of rats rendered diabetic throughout pregnancy demonstrate vascular dysfunction. The effects of the development of diabetes later in pregnancy (gestational onset diabetes) on vascular function in the offspring are not known. **Hypothesis:** Female offspring of mother rats made diabetic during the latter half of pregnancy demonstrate altered vascular reactivity and endothelial dysfunction. **Methods:** Pregnant rats were made diabetic by administration of streptozotocin (50 mg/kg ip) on day 12 of gestation. Insulin was administered subcutaneously twice each day to maintain glucose levels of 100 - 400 mg/dl and avoid ketoacidosis. Control rats received injections of saline. All rat pups were cross-fostered after birth to non-diabetic dams. Offspring (n = 7, each group) were sacrificed at 6-8 mo of age and aortic vascular reactivity assessed by wire myography. Data were assessed by ANOVA. **Results:** Birthweights and postnatal growth were similar between the offspring of control and diabetic dams. Endothelium-intact aortas from offspring of diabetic rats showed significantly ($p < 0.05$) enhanced contractility to KCl, endothelin-1 and noradrenaline, but not angiotensin II or serotonin. Co-administration of L-NNA (a nitric oxide synthase inhibitor) normalized the responses to endothelin-1 and noradrenaline. Relaxation to acetylcholine (endothelium dependent) but not nitroprusside (endothelium independent) was also significantly impaired in offspring of diabetic rats. **Conclusions:** Adult offspring of gestational diabetic rats displayed altered vascular responses and endothelial dysfunction. Alterations in endothelial function may provide a mechanistic link between intrauterine exposure to diabetes and the later development of cardiovascular disease.