

Expression of the *APC* Tumor Suppressor Gene is Regulated during Mammary Gland Development and Differentiation

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Background and Purpose: With almost 270,000 new cases of breast cancer and over 40,000 deaths, breast cancer is one of the greatest health concerns facing women. The *APC* tumor suppressor gene has been shown to be inactivated in as many as 40% of sporadic breast cancers, and mice carrying a germline mutation in *Apc* are predisposed to mammary tumors. Our laboratory has shown previously that APC expression is induced during pregnancy and lactation in the mouse mammary gland, and *Apc*-deficiency results in defective lobulo-alveolar development. The current project tests the hypothesis that APC expression is regulated by lactogenic hormones both *in vitro* and *in vivo*. **Methods:** In the *in vitro* study, we exposed Eph4 mouse mammary epithelial cells to 10 nM 17 β -estradiol, 10 nM 17 β -estradiol with 100 nM progesterone, 50 ng prolactin in saline or vehicle (sesame seed oil with saline) for 4, 24 or 48 h. Total RNA was harvested from the cells. APC and GAPDH (as a normalization control) were amplified using Reverse-transcriptase (RT) real-time PCR to quantify gene expression. For the *in vivo* studies, ovariectomized C57BL/6 (strain) mice (n=7/group) were treated with daily injections of 1cc for 20 days. Mammary tissue was harvested and RNA was isolated. APC and GAPDH gene expression were evaluated by real-time RT-PCR. **Results:** Eph4 cells exposed to lactogenic hormone for 4 h demonstrated an increase in APC expression, particularly in the 17 β -estradiol and progesterone treated cells, compared to those treated with vehicle. At 24 and 48 h there were no obvious differences in APC expression between the treatment groups. Additionally, we observed that mammary tissues from mice treated with estradiol and progesterone had increased APC mRNA expression compared to those mice treated with vehicle or estrogen alone. **Conclusions:** These data suggest that expression of the *APC* tumor suppressor gene is regulated by lactogenic hormones in the mammary gland, and support a model in which APC is an important regulator of mammary gland function during pregnancy and lactation.

Neuronal Nitric Oxide Synthase (nNOS) Is Not Involved In Fibroblast Growth Factor 2 (FGF2) – Induced Cardioprotection In A Murine Model of Ischemia-Reperfusion Injury

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Introduction. Ischemic heart disease caused 20% of all deaths in 2003 and is the single largest killer of Americans. Our laboratory showed previously that over-expressing FGF2 is cardioprotective during ischemia-reperfusion injury. There are three isoforms of NOS (all three are found in heart), and nNOS is implicated in modulating cardiac function. Since certain biological actions of FGFs can occur via NO signaling, we investigated the potential link between FGF2-induced cardioprotection and activation of nNOS during ischemia-reperfusion injury. **Methods.** Wild type (Wt) and two lines of transgenic (Tg) mouse hearts which over-express FGF2 were connected to a work-performing heart apparatus and perfused with an oxygenated Krebs-Henseleit solution. After 30 minutes equilibration, hearts were subjected to ischemia (60 minutes) followed by reperfusion (120 minutes). nNOS activity was inhibited with 1-(2-trifluoromethyl-phenyl) imidazole (TRIM, 100 μ M) dissolved in ethanol (Vehicle) prior to ischemia and reperfusion. Functional data, perfusate gases, and coronary effluent were obtained at specific time points. Nitrite concentrations in effluents were measured with a fluorometric assay. **Results.** No significant differences were observed in recovery of contractility between vehicle-treated or TRIM-treated hearts in either group (Wt-Veh 69.5%, Tg-Veh 68.0%, Wt-TRIM 51.8%, Tg-TRIM 76.5%). No differences in rates of relaxation were observed between the groups. No differences in NO levels were observed in Wt hearts. NO levels were significantly lower after 120 minutes reperfusion for vehicle and TRIM-treated Tg hearts. Preliminary data indicate that blocking nNOS leads to improved recovery of contractile function in Wt and Tg male hearts. **Conclusions.** FGF2-induced cardioprotection does not rely on nNOS-associated pathways. nNOS-derived NO may be deleterious to male hearts during ischemia-reperfusion injury.

Functional and Morphologic Changes in Anterior Limbic Structures are Observed in Bipolar Disorder

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Bipolar disorder (BPD) is a psychiatric illness characterized by cycling between episodes of mania, euthymia and depression. Its dynamic and lifelong expression suggests that it is the result of a dysregulation of the neural systems that maintain emotional homeostasis. Recent advances in neuroimaging techniques have implicated components of the anterior limbic system (ALS) in the disease but have done so incompletely. A better understanding of the dysfunctional neural circuits within the ALS could influence the development of more targeted drugs and improve the diagnosis and treatment of BPD.

Methods: We obtained structural and functional MRI scans from 32 bipolar and 19 healthy participants while they performed a behavioral task that elicits both emotional and attentional brain activation patterns. Voxel based morphometry (VBM) was used to determine whether differences in gray and white matter density could be used to explain differences in blood flow observed during fMRI. VBM was also used to compare density patterns between patients with more and less severe symptoms.

Results: Distinct differences in density were observed in several areas of the anterior limbic system including the posterior cingulate cortex, thalamus and caudate. There were no statistically significant differences in white matter. Both functional and structural abnormalities were observed in the left thalamus. Additionally, patients with more severe symptoms showed a greater reduction in gray matter density in the anterior and posterior cingulate cortex, right caudate and thalamus compared to patients with more mild symptoms.

Conclusions: The presence of both structural and functional abnormalities in the left thalamus suggest that its dysfunction may play a more central role in the development of bipolar disorder than was previously considered. More extensive involvement of the cingulate cortex, caudate and thalamus in patients with higher YMRS scores could be explained by a more dynamic model of the brain in BPD. For

example, environmental triggers or developmental processes could produce structural changes in the brain that exacerbate the emotional and behavioral deficits observed.

Intestinal Expression of Interleukin-9 Induces Mastocytosis and Mast Cell Activation

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Background: Clinical and experimental investigations have demonstrated a link between Th2 intestinal inflammation, antigen-specific IgE, and mast cell derived secondary mediators including histamine, serotonin, and platelet-activating factor (PAF) in the effector phase of food-induced anaphylaxis. The Th2-cytokine IL-9 is thought to play a central role in mastocytosis and mast cell activation.

Hypothesis: Overexpression of IL-9 in the intestine induces intestinal mastocytosis and also induces the expression of mast cell genes including mast cell protease (MCP) -1, -2, -4, and -5, and also the high affinity IgE receptor FCεR1α.

Methods: Jejunum segments were obtained from wild-type (WT) and IL-9 transgenic (TG) mice. Intestinal mast cell levels were determined by staining for chloroacetate esterase activity. cDNA was generated from jejunum RNA using standard techniques. Quantitative RT-PCR was performed on cDNA using primers specific for mouse mast cell protease 1, 2, 4, and 5, and also FCεR1α, the high affinity IgE receptor, and the housekeeping gene GAPDH.

Results: Ectopic overexpression of IL-9 in the intestine was associated with intestinal mastocytosis (12.63 ± 2.80 vs. 0.48 ± 0.24 mast cells/hpf, iFABP-IL-9TG vs WT mice; $p < 0.05$). Levels of mMCP-1 (34.47-fold increase, $p=0.005$), mMCP-2 (97.12-fold increase, $p < 0.005$) mMCP-4 (10.09-fold increase, $p < 0.05$) and FCεR1α (4.57-fold increase, $p < 0.05$) expression in the jejunum of IL-9 TG mice was significantly elevated as compared to WT mice. In contrast, no significant difference in intestinal expression of mMCP-5 (1.5-fold increase, $p=1.0$) was observed in IL-9 TG mice as compared to WT mice.

Conclusions: These data establish that intestinal expression of IL-9 induces mastocytosis and the expression of the mast cell related genes,

mMCP-1, -2, -4 and FCεR1α. These studies suggest that IL-9 may play an important role in food-induced intestinal anaphylaxis.

Domain analysis of transcription factors Pu.1 and SpiC during Bcell development.

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PU.1 and Spi-C are both Ets transcription factors active during B cell development. PU.1 and Spi-C have homologous DNA binding domains, however their activation domains are divergent. PU.1 is critical for B cell and macrophage development from stem cells, and is expressed throughout B cell development. Spi-C is expressed selectively during B cell development and in mature macrophages, but its functions are still unclear. PU.1 and Spi-C can bind to identical DNA recognition sites in target genes, but have opposing biological activities. For the IgH gene, PU.1 acts as a repressor while Spi-C acts as an activator. For the Fc R IIb gene, PU.1 acts as an activator while Spi-C acts as a repressor. *We hypothesize* that differences in transcriptional regulatory activity between PU.1 and Spi-C are due to different N-terminal activation domains, with no difference arising from the conserved C-terminal domains. To test this hypothesis we constructed chimeras, swapping the N-terminal domain of PU.1 with that of Spi-C and vice versa. *We expect* that when expressed in pro-B cells, a chimeric protein with a Spi-C DNA-binding domain and a PU.1 activation domain will behave identically to PU.1 (increase Fc R IIb, decrease IgH). Conversely, we expect that a chimeric protein with a PU.1 DNA binding domain and a Spi-C activation domain will behave identically to Spi-C (decrease Fc R IIb, increase IgH). The results of these experiments are pending; these proteins will be expressed in pro B-cell 38B9 cell lines, along with luciferase reporters linked to transcription of the IgH intronic enhancer or Fc RIIb promoter.

Quantitative Analysis of Small Intestinal Length Using Radiologic Images

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Background

Short Bowel Syndrome (SBS) is characterized by the functional or anatomic loss of extensive segments of small bowel, resulting in massive reduction of the absorptive capacity. The major correlate to patient survival is the length of remaining bowel. Currently, no conventional method exists by which remaining bowel length can be accurately measured. Our goal was to use a mouse model, utilizing computerized tomography scanning, to determine bowel length *in situ*.

Methods

Mice were fed a 1:1 dilution of water-soluble contrast in liquid food for 90 minutes following ad lib water only overnight. Under general anesthesia, CT scans were performed on unoperated mice. Small bowel was harvested from pylorus to cecum; *in vivo* length was measured using the laboratory's established length standardization protocol. Small bowel length was measured *in situ* using CT images created on a Vitrea workstation. *In situ* measurements were correlated with *in vivo* lengths.

Results

Comparing length measurements obtained via harvest and radiologic imaging, a R^2 of 0.847 was achieved with an n=6. There is a trend of bowel measurements approaching acceptable correlation values between *in vivo* and *in situ* environments.

Discussion

Measuring bowel length *in situ* was a challenge likely heightened by the limitations of current commercial computer software; convolutions of the bowel created the dilemma of measuring segments within repeated image slices that were unable to be properly interpreted by the software. Therefore, the current CT measurements are more of an approximation than a specific length. However, the utility of a system like this is still very high. Future work will include increased proficiency with the software, and refining the methods by which the bowel is measured. Improving our ability to provide accurate *in situ*

measurements will be widely applicable to the SBS population and allow exceedingly improved long-term patient management and surgical decision-making.

Regulation of Neuronal Calcium Oscillations by AMPA and NMDA Receptors

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Learning and memory are widely thought to be controlled by changes in synaptic strength. Calcium signaling is known to be required for many of these changes and calcium oscillations have been suggested to be especially important. Networks of cortical neurons have been shown to undergo spontaneous calcium oscillations as a result of the release of the neurotransmitter glutamate. Glutamate in turn activates two distinct ion channels, AMPA receptors for Na⁺ influx and NMDA receptors for Ca²⁺ influx.

Since it is not known what the relative influence of these two glutamatergic receptors is on calcium oscillations, we felt it would be important to determine how the activation of each receptor affects the characteristic features of calcium oscillations. We therefore measured the frequency, amplitude, and basal calcium concentrations of spontaneously oscillating rat cortical neuronal networks using the calcium-sensitive fluorescent dye fura-2.

We found that addition of glutamate to neuronal cultures yielded a complex response, particularly on the frequency of Ca²⁺ oscillation frequency. On the other hand, activation of AMPA receptors alone resulted in a simpler response with a slightly increased Ca²⁺ baseline, a decreased oscillation amplitude, and a decreased frequency. In contrast, activation of NMDA receptors alone caused a much larger increase in Ca²⁺ baseline, a decreased amplitude similar to AMPA, but an increased frequency. Thus, NMDA and AMPA act in opposing manners on the frequency of calcium oscillations. We also found that blocking the action of NMDA receptors, either by addition of the antagonist MK-801 or by inhibition of the synthesis of the NR1 subunit of the receptor with siRNA, markedly reduced the frequency of oscillations. We present a synaptic model suggesting a mechanism by which AMPA and NMDA may exert their effects.

There is a considerable body of evidence suggesting that large increases in postsynaptic calcium cause synaptic potentiation whereas

small increases result in synaptic depression. Because AMPA and NMDA receptors have opposing effects on oscillation frequency, our data suggest that one way in which cells may regulate their calcium levels and thereby regulate their synaptic strength is via the ratio of their AMPA to NMDA receptors.

Interaction between CFTR and Cl/HCO₃⁻ Exchanger SLC26A6 is Essential for HCO₃⁻ Secretion in the Intestine

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Background: Approximately 30,000 people in America are affected by cystic fibrosis. In this autosomal recessive disorder, in which a mutant form of the cystic fibrosis transmembrane conductance regulator (CFTR) becomes inactivated, there are multiple clinical manifestations, the most severe being thickening of the mucous of the lung and inability to produce or deliver bicarbonate and enzymes in the digestive system. CFTR is an apical anion channel that mediates secretion of Cl⁻ into the lumen from the cell, and subsequently drives HCO₃⁻ secretion via activation of Cl⁻/HCO₃⁻ exchange. SLC26A6 is the primary apical chloride/bicarbonate exchanger in the small intestine, and is also present on the apical membrane of the pancreatic duct and the kidney proximal tubule. It has been previously suggested that SLC26A6 and CFTR are linked through their PDZ binding domain.

Objectives:

- 1) Confirm that both CFTR and SLC26A6 are located on the apical membranes of enterocytes in the mouse and rat duodenum.
- 2) Describe the interaction between CFTR and SLC26A6, and show that they are physically bound to one another.

Methods: Tissue sections of mouse and rat duodenum were prepared and single immunofluorescent labeling with either CFTR or SLC26A6 specific antibodies was performed. Separately, rat duodenum enterocytes were obtained, lysed, and then immunoprecipitated with CFTR antibodies. This immunoprecipitate was then analyzed using Western blot with antibodies for SLC26A6. In addition, the supernatant from the immunoprecipitation reaction was also analyzed with Western blot and antibodies for SLC26A6 to see if SLC26A6 was present.

Results: Sections of both mouse and rat duodenum displayed fluorescent labeling on the apical surface of enterocytes when either SLC26A6 or CFTR antibodies were used. The immunoprecipitation from rat enterocytes and subsequent Western blot of the

immunoprecipitate yielded a band at approximately 70kD. This band correlates to the location of the SLC26A6 protein. The supernatant yielded faint lines in the 70 kD range.

Conclusions and Significance: With both CFTR and SLC26A6 located on the apical membrane of enterocytes, these results demonstrate physical interaction between the two transport proteins. This conclusion is based on the above results indicating that CFTR causes the immunoprecipitation of SLC26A6 protein, which was verified by Western blot analysis using SLC26A6 antibodies. These results strongly suggest that CFTR and SLC26A6 are linked and bound together. If there is similar binding between these two proteins in humans, as in the rat duodenum, and SLC26A6 is dependent on this interaction with CFTR, then this may provide an explanation for the bicarbonate secretion defect in various epithelia in cystic fibrosis patients when a mutant protein may not allow binding to SLC26A6.

Reference values for bone mass and density of the lumbar spine for children 6 to 36 months of age.

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Dual-energy x-ray absorptiometry (DXA) is widely used for measuring bone mineral content (BMC) and density (BMD) to aid the assessment of bone health in children. Currently, there are no reference values of bone mass and density for children 6 to 36 months of age that can be used as a standard for comparison. The aims of this project were: 1) to recruit 200 healthy children 6 to 36 months of age (100 boys, 100 girls); 2) to determine if there are age and sex differences in BMC and BMD of the lumbar spine; and 3) to develop age-specific BMC and BMD reference values based on current generation DXA technology. Eligibility criteria were weight and length between the 5th to 95th percentiles for age, normal gross motor skill attainment, and absence of health conditions known to affect BMC. A DXA scan of the lumbar

spine was obtained by a Hologic QDR4500A densitometer, and analyzed by software version 12.4 to give measurements of bone area, BMC and BMD. A total of 52 subjects were recruited (27 girls, 25 boys), a DXA scan was not obtained on 4 subjects because of intense crying, and a “usable” DXA scan (no movement) was obtained on 37 subjects. The likelihood of a “usable” scan increased with age: 57% of infants 6-18 months of age vs. 88% of infants 18-36 months of age. Bone area, BMC and BMD increased linearly with age ($P < 0.001$). Bone area and BMC were smaller in girls than in boys ($p < 0.001$), but there was no sex difference in BMD. Reference ranges for bone measures should be developed separately for boys and girls and should be age specific. The utility of developing reference DXA data for infants < 18 months of age is questionable.

Multiparametric Imaging of Bone Architecture: A Pilot Study

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Background: Conventional measurements, such as bone mineral density (BMD) analysis, are imperfect predictors of osteoporotic fractures. 3-D bone architecture is a major determinant of bone strength. New technologies such as magnetic resonance microimaging (μ MRI) permit in-vivo assessment of 3-D bone architecture at peripheral sites. Thus MRI derived parameters hold promise for improved risk prediction, fracture evaluation, and monitoring the response to therapeutic treatments.

Purpose: To establish feasibility of whole body and ultra-high resolution specimen CT and MR imaging, in order to investigate the correlation between vertebral fracture status and multi-site trabecular bone micro-architecture.

Methods: One deceased female was imaged with dual energy CT within 2 days of expiration. Subsequently, the cadaver was imaged with multi-parametric 1.5T MRI with additional ultra-high resolution of the spine and distal radius. The cadaver was then sectioned and ultra high resolution specimen imaging obtained with both 7T μ MRI and μ -CT.

Results: From the CT and MR whole body images we are able to determine that there were no osteoporotic fractures present. At 1.5T we were able to achieve an in-plane resolution of 195x195 microns. 7T μ MR and μ CT depicted specimen trabecular bone micro-architecture with spatial resolution reaching 74x74 microns and 50x50 microns, respectively.

Conclusion: We demonstrated the feasibility of correlative whole body and ultra high resolution bone specimen CT and MR imaging. At 1.5T, we were able to assess fracture status and trabecular architecture. At 7T we were able to attain higher resolution images approaching the “gold standard”, μ CT. Currently, a five cadaver study is underway, using the protocols developed from this project to look at a number of parameters involving trabecular 3-D bone micro-architecture. If the different sites show a correlation in bone micro-architecture, then distal sites which are more easily imaged in-vivo, can be used as a biomarker to predict overall trabecular bone micro-architecture, and thus fracture risks in patients.

The Role of Cholesterol and Fatty Acids on Intestinal Apo A-IV Synthesis and Secretion

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Background: Apo A-IV is one of the major apolipoproteins that is synthesized and secreted in response to the absorption of lipid by the small intestine. Apo A-IV is proposed to be involved in the formation and secretion of chylomicrons, lipoprotein metabolism, control of food intake, gastric function, prevention of atherosclerosis, and preventing the oxidation of lipoproteins. Previous studies demonstrated that Apo A-IV synthesis and secretion is stimulated by the absorption of long chain fatty acids, but not short chain fatty acids, through the formation of chylomicrons.

Hypothesis/Aims: We hypothesized that cholesterol absorption stimulates Apo A-IV synthesis and secretion by the small intestine. The aim of this project was to infuse medium chain fatty acids, with and without cholesterol added, into the duodenum of lymph fistula rats to determine if Apo A-IV synthesis and secretion was stimulated by cholesterol. Compared with the infusion of medium chain fatty acids alone, which do not stimulate chylomicron formation, it can be determined if cholesterol will stimulate Apo A-IV synthesis and secretion.

Methods: Two groups of non-bile diverted lymph fistula rats (n=7 in each group) were studied. The control group received medium chain fatty acid without cholesterol. The experimental group received medium chain fatty acid with cholesterol. Lymph samples were collected before the infusion, and then hourly for 8 hours. The lymph was sampled for Apo A-IV, triglyceride, and cholesterol content.

Results: The lymphatic Apo A-IV output did not differ significantly between rats infused with medium chain fatty acids with and without cholesterol ($p > 0.05$, not significant for all time points tested).

Conclusion: Cholesterol absorption is not a signal for the stimulation of Apo A-IV synthesis and secretion by the small intestine.

Relationship of Body Mass Index Rebound Age and Adverse Cardiovascular Risk Factors in Children

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Purpose: In early childhood BMI reaches a nadir, the BMI rebound, before increasing through later childhood, adolescence, and adulthood. While earlier age of BMI rebound is associated with obesity and obesity-related disease, it is unknown if the timing of BMI rebound is associated with the presence of adverse cardiovascular risk factors in childhood. The aim of this study was to determine if timing of Body Mass Index (BMI) rebound is associated with the presence of adverse cardiovascular risk factors in children at age 7 years. **Methods:** This analysis was performed on data obtained from children participating in a prospective cohort study. BMI values were recorded every four months for four years for each child. At age 7 years the following variables were obtained: systolic and diastolic blood pressure, serum insulin, leptin, lipid profiles, and echocardiographic assessment of left ventricular and left atrial function and geometry. The cohort was divided into three groups based on age of BMI rebound: 1) early BMI rebound age (below the 25th percentile), 2) middle BMI rebound age (between the 25th and 75th percentiles), and 3) late BMI rebound age (above the 75th percentile). **Results:** Mean early and late BMI rebound ages were 4.4 and 6.6 years for boys, and 4.2 and 5.7 years for girls respectively. In both genders, earlier age of BMI rebound was associated with higher BMI and systolic and diastolic blood pressures, higher serum insulin and leptin levels, and higher left ventricular mass and left atrial size at 7 years of age. **Conclusions:** Earlier age of BMI rebound is associated with adverse cardiovascular changes including higher blood pressure, elevated insulin and leptin levels, and left ventricular hypertrophy and left atrial dilatation. This study supports the clinical monitoring of cardiovascular risk factors in children who demonstrate early age of BMI rebound in order to manage and prevent cardiovascular disease.

Stage-of-Change Assessment in the Parents of Pediatric Emergency Department Patients with Unrecognized Mental Illness

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Introduction: Despite its prevalence, pediatric mental illness often goes untreated. Little is known about the reasons for this lack of treatment, but one factor may be related to the family's readiness to seek treatment for the child. We explored the stages of readiness to seek treatment in families of children screening positive for previously undiagnosed mental health problems and how stage was related to global impairment of the child.

Methods: Children without a history of mental health problems who presented to the emergency department for urgent but stable medical complaints were approached for participation in the study. Of these thirty-one were screened for a mental health problem using the DISC Predictive Scales (DPS). Twenty-four children screened positive for at least one mental health problem as reported by the child or their parent. Families also completed the University of Rhode Island Change Assessment Scale (URICA). Impairment was determined by the Children's Global Assessment Scale (C-GAS).

Results: Overall children were more likely to endorse items in the precontemplation stage of readiness to seek treatment compared to their parents, who were more likely to endorse items in the contemplation, action, and maintenance stages. Endorsing items in the contemplation stage was highly correlated with endorsement of items in the action and maintenance stages. Children who were not impaired by their mental health problems were likely to endorse items in the precontemplation stage.

Conclusions: Differences in readiness to seek treatment appear to exist between children and their parents and between impaired and unimpaired children. Furthermore some stages appear to be strongly associated with others. While more research subjects are needed, these results have implications for the future use of stage-specific

interventions in the ED and greater engagement in mental health treatment.

Selective Serotonin Reuptake Inhibitors and Risk of Hemorrhagic Stroke: A Population-Based Case-Control Study

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Background: Selective Serotonin Reuptake Inhibitors (SSRI) are among the most widely prescribed classes of drugs. Several reports have observed an increased bleeding risk (gastrointestinal, respiratory, vaginal, and cerebral) associated with SSRI use, which is suspected to be due to their antiplatelet effect. In addition to its effects in the brain, serotonin is also used by platelets as a signaling molecule in the promotion of clot formation. SSRIs have been shown to decrease serotonin receptor activity, serotonin levels, and uptake in platelets. We tested the hypothesis that SSRIs increase the risk for hemorrhagic stroke and potentiate the risk of hemorrhagic stroke associated with antiplatelets and anticoagulants.

Methods: Cases of intracerebral (ICH) and subarachnoid hemorrhage (SAH) in the Greater Cincinnati region from 5/97 to 10/05 were identified by screening all area hospital admissions, emergency room logs, and discharge ICD-9 codes. Patients were approached for enrollment in a genetic sampling and interview arm. Subjects who agreed were matched by age, race, and gender to population-based controls. Medical records were reviewed for risk factors and medication use, including SSRIs, antiplatelet agents, anticoagulants, and statins. Logistic regression was used to determine the risk of significant factors to hemorrhagic stroke and subtypes of hemorrhagic stroke.

Results: Total enrollment included 916 patients with hemorrhagic stroke of which 71 (7.7%) were on an SSRI at the time of stroke and 1,776 age-, race-, and gender-matched controls of which 158 (8.8%) were on an SSRI. After controlling for smoking, hypertension, frequent alcohol use, heart disease, prior ischemic stroke, BMI, hypercholesterolemia, statin use, and education level, SSRI use was not independently associated with increased risk for hemorrhagic stroke ($p = 0.25$). Analysis of ICH and SAH separately revealed no increased risk of SSRI use for ICH (OR= 1.1 CI: 0.7-1.8; $p=0.63$) or SAH (OR= 0.6 CI: 0.4-1.0; $p = 0.054$). In multivariate analysis, the use of SSRIs concomitantly with warfarin was not associated with a significantly

greater risk of hemorrhagic stroke (OR = 4.7 CI: 1.2-18.4) than warfarin alone (OR= 3.0 CI: 1.8-5.0). Similarly, the use of SSRIs concomitantly with antiplatelets was not significantly greater in risk of hemorrhagic stroke (OR=0.8 CI: 0.5-1.5) than antiplatelets alone (OR=1.1 CI: 0.9-1.3).

Conclusion: Despite reports that SSRI use increases the risk of bleeding complications, we did not find an independent association of SSRI use with hemorrhagic stroke, ICH, or SAH in a large population-based case-control study. In addition, potentiation of risk with aspirin or warfarin was not observed. SSRI use does not appear to lead to significant risk of hemorrhagic stroke.

Improved Engraftment of Bone Marrow and Mobilized Peripheral Blood Stem Cells in a Fanconi Anemia Murine Model.

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Fanconi anemia (FA) is a genetic syndrome characterized by development of progressive bone marrow failure and cancer predisposition. The difficulty in harvesting FA HSC and their fragility during subsequent *in vitro* manipulations has proven a confounding factor in attempted gene therapy of this disease. Mobilization of HSC/P in FA patients is poor (Croop *et al.* Blood, 2001) probably due to HSC deficiency. We have previously shown that the genetic deletion of the Rac GTPases 1 and 2 results in an increase in circulating hematopoietic stem cells and progenitors (HSC/P) (Gu *et al.* Science 2003). In addition, administration of a single dose of a small molecule inhibitor of Rac GTPases, NSC23766, results in a transient mobilization of engraftable stem cells (Cancelas *et al.* Nat. Med., 2005). Here, we analyzed the role of NSC23766 in mobilizing HSC/P in FA A (*Fanca*^{-/-}) mice (Cheng *et al.*, Hum. Mol. Genet., 2000; kindly provided by M. Grompe, OHSU). First, we validated that this murine model of FA demonstrated a stem cell phenotype by a competitive repopulation assay of BM HSC. We found that *Fanca*^{-/-} HSC contribute decreased chimerism in short-term engraftment (52.6 ± 2.6% donor engraftment) compared to wild-type (WT) controls (63.8 ± 1.0%, respectively, $p < 0.005$). BM and spleen homing of *Fanca*^{-/-} HSC/P at sixteen-hours post infusion was not impaired (7.0% in BM and 6.1% in spleen) compared to WT mice (7.8% in BM and 5.4% in spleen) and there was no difference in expression of CXCR4, α_4 -integrin, α_5 -integrin and L-selectin between Lin⁻/c-kit⁺/Sca-1⁺ BMC and mobilized PBC derived from *Fanca*^{-/-} and WT mice, also supporting an intrinsic HSC defect. We then analyzed the ability of NSC23766, alone or in combination with G-CSF, to mobilize HSC. We observed that *Fanca*^{-/-} mice also show an impaired mobilization response to G-CSF administration (200 mcg/Kg/day for five days), which can be partially rescued by administering a single dose of NSC23766, 6 hours before peripheral blood harvest (Table1). We additionally demonstrated the impaired

engraftment of *in vitro* manipulated *Fanca*^{-/-} BMC in a competitive transplant assay. This engraftment defect could be completely ameliorated by treatment with Diprotin A (5.9±2.0% donor engraftment untreated vs. 12.0±4.4% treated; p value = 0.01). Diprotin A is an inhibitor of CD26 peptidase which has been shown to cleave SDF1alpha/CXCL12. The combined use of G-CSF and NSC23766 may constitute a future novel approach to induce mobilization of Fanconi anemia HSC and, when coupled with Diprotin A treatment, could act to enhance the engraftment of cells undergoing genetic correction.

	Short Term Engraftment (+1 month)	Long Term Engraftment (+4 months)
WT GCSF (%)	100 ±34.08	100 ±52.34
WT GCSF + NSC 23766 (%)	77.43 ±37.11	53.34 ±54.61
<i>Fanca</i> ^{-/-} GCSF (%)	11.25 ±5.92 *	2.54 ±2.59 **
<i>Fanca</i> ^{-/-} GCSF + NSC 23766 (%)	24.45 ±1.89	39.01 ±29.47 ***

* p value < 0.05, compared to WT GCSF; ** p value < 0.001, compared to WT GCSF, *** p value < 0.05 compared to *Fanca*^{-/-} GCSF

Energy Balance is Prospectively Related to Change in Abdominal Fat Accumulation among Overweight Children

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Introduction: Childhood obesity is an increasing public health problem in the United States. Visceral fat deposition, in particular, has been linked to an increased risk of diabetes, heart disease and stroke in adults. Few studies have looked at factors that contribute to visceral fat accumulation in children.

Objective: The objective was to determine whether there were any links between diet composition and visceral fat accumulation.

Methods: Forty-two children within 3 months of their 8th birthday were recruited from the Greater Cincinnati area (n=36 completed all assessments). All children were above the 75th percentile of BMI for their age and sex. Children were evaluated every six months for 2 years in this observation study. At each time point, children (with the help of a parent) kept a 3-day food log. Accelerometers were used for 7 days to monitor physical activity. Visceral(VAT) and subcutaneous abdominal (SAT) fat were evaluated using MRI scans of 10cm in the abdomen. Longitudinal analysis using SAS Proc Mixed procedures examined whether dietary factors, both by themselves and when adjusted for physical activity, were dynamic correlates of the change in children's VAT.

Results: VAT, SAT, and total body fat increased linearly and significantly over the 2-year time period. Reported caloric intake did not significantly change, but physical activity declined, so physical activity-adjusted calories increased over time. There was no evidence of relations between absolute caloric intake or any macro-nutrient and whole body, VAT, or SAT. However, calorie intake adjusted for physical activity was found to be positively correlated to VAT (p=0.0114) and SAT (P<0.0188). Change in physical activity-adjusted caloric intake was not related to change in total body fat. Unexpectedly, a negative correlation was found between calorie intake when controlled for the child's body weight and subcutaneous adipose tissue (P<0.0001) and visceral adipose tissue (P=0.0008).

Summary and Conclusion:

Greater calories relative to physical activity among overweight children appears related specifically to the accumulation of abdominal fat. There appears to be little evidence of relations between specific macronutrients and either whole body or compartments of abdominal fat, although our unexpected findings regarding weight-adjusted calories and whole and abdominal fat highlight the potential limitations of child dietary reports.

Total Pancreatectomy and Autologous Islet Cell Transplantation as a Means to Treat Severe Chronic Pancreatitis

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BACKGROUND: Chronic pancreatitis causes pain episodes frequently requiring hospitalization and severely compromising quality of life. For patients who suffer from severe chronic pancreatitis, total pancreatectomy followed by autologous islet cell transplantation (AIT) can alleviate pain and preserve endocrine function. **STUDY DESIGN:** From 2000 to 2006, 75 patients underwent pancreatectomy with AIT at the University of Cincinnati. Patient demographics, narcotic requirements (standardized by conversion to morphine equivalents), and insulin dependence were recorded prior to surgery and at subsequent follow-up visits. Statistical analysis was performed to determine whether patient narcotic requirements decreased following surgery as well as factors associated with insulin independence. **RESULTS:** Seventy-five patients (50 women, 25 men) with a mean age of 37.5 years underwent completion ($n = 24$), total ($n = 47$), or partial ($n = 4$) pancreatectomies with AIT. The average number of islet equivalents harvested was 413,324 (range 4,100 to 1,065,600). All patients had preoperative pain and had been taking opioid analgesics. There was a notable reduction in narcotic usage ($p < 0.001$) from the mean preoperative requirement of 249.5 MEs to 98.7 MEs at last follow-up. Fifty-one percent of patients are currently narcotic independent. At last follow-up, the mean units of insulin required per day was 16.4 (range 0 to 80). Twenty-eight percent are insulin independent and 17% require sliding scale insulin (1-9 units/day). Of the 34 patients that require less than ten units of insulin per day, 31 are female, a significant finding ($p < 0.001$). These patients also were prescribed less insulin upon discharge from the hospital than patients currently requiring greater than ten units of insulin per day (10 units/day vs. 20 units/day; $p < 0.001$). **CONCLUSIONS:** Pancreatectomy with AIT can alleviate pain for patients with chronic pancreatitis and preserve endocrine function. Factors associated with insulin independence include female gender and insulin prescribed at patient discharge from the hospital.

Characteristics of Women's Health Electives in Medical Schools and Factors Influencing Their Development

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Background: Medical educators recognize the importance of integrating education about women's health into the medical school curriculum. The Association of American Medical Colleges (AAMC) in collaboration with the Office of Research on Women's Health (ORWH) at the National Institutes of Health (NIH) has verified the lack of women's health education in medical schools, while the Association of Professors of Gynecology and Obstetrics (APGO) along with the Association of Reproductive Health Professionals (ARHP) have provided guidelines to aid in developing women's health educational programs. However, medical schools continue to face obstacles in developing women's health electives. The aim of this study was to identify how many U.S. and Canadian medical schools currently offer women's health electives, describe the characteristics of these electives, and identify key factors contributing to the development and sustainability of women's health electives.

Methods: Medical educators from all U.S. and Canadian medical schools (N=126) identified through a list provided by the AAMC, and medical educators contacted by mail and through medical educator listservs and were asked to respond to an online survey assessing electives in women's health offered by their medical school. The survey included items that assessed whether women's health electives were offered at their institution, and whether there was a plan to offer such electives in the future. If electives were offered, additional items assessed the specific characteristics of each women's health elective offered and the impetus, facilitating factors and barriers to establishing these electives. Descriptive analyses were performed using SPSS v.14.0.

Results: Of the 126 medical schools surveyed, a total of 73 medical schools responded (58%) and 56 (44%) indicated that they offered at least one women's health elective. The majority (13, 54.2%) indicated that they offered only one women's health elective and 15 (47%)

offered enrollment to only one student per session. Respondents indicated that they used APGO (13, 43%) and ARHP guidelines (6, 20%) to aid in elective development. Most electives were available to fourth year students (31, 94%), were available to visiting students (29, 90%), required student evaluations (22, 79%), had no on-call requirements (28, 90%), and were offered in an outpatient setting (70% of time spent). Obstetrics/gynecology was indicated by 17 (52%) as the sponsoring department. The majority of preceptors (26, 90%) were provided by the department of obstetrics/gynecology. The impetuses for elective development mentioned most frequently were student interest (22, 67%) and faculty interest (18, 55%). The facilitating factors mentioned most frequently were also student interest (18, 55%) and faculty interest (31, 94%). Few noted grant funding (2, 6.1%) as a contributing factor for development. A major barrier to elective development was time pressure (22, 81.5%).

Conclusions: Cultivating interest in women's health among students, offering incentives for faculty to address women's health, providing funding support for the development of women's health electives, and encouraging multidisciplinary involvement in women's health education are needed for further development of women's health electives and further incorporation of women's health into the medical school curriculum.

Activated Guanylate Cyclase C and its Effects on Intestinal Cancer Cell Proliferation

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BACKGROUND: There is an inverse relationship between the worldwide incidence of colon cancer and infection with enterotoxigenic *Escherichia coli* (ETEC). ETEC produce a heat stable enterotoxin agonist of the intestinal epithelial receptor guanylate cyclase C (GC-C). Although the GC-C signaling pathway mediates intestinal secretion, it also plays a role in intestinal epithelial cell (IEC) proliferation and perhaps cancer susceptibility. Based on studies that found a loss of endogenous GC-C ligands guanylin (Gn) and uroguanylin expression in adenomas as well as elevated IEC proliferation in Gn-null mice, it was hypothesized that activation of GC-C inhibits IEC proliferation. We investigated the effects of GC-C activation on proliferation in both a non-transformed cell line (IEC-18) and a colon cancer cell line CaCo-2 BBE (BBE) as well as involvement of cell cycle regulatory molecules ERK, PKC- α and Retinoblastoma (Rb). **METHODS:** IEC-18 cells lack endogenous GC-C, and thus were transduced with a retroviral construct expressing GC-C. Experimental groups of the transduced IEC-18 cells, BBE cells, or PKC- α inhibited BBE cells were stimulated with Gn and/or serum. Cell numbers and proliferation was measured via tetrazolium salt metabolism and ELISA-based BrdU incorporation assay. Western blotting was used to measure phosphorylated ERK and phosphorylated Rb. **RESULTS:** IEC-18 cells did not respond to GC-C activation with respect to cell cycle changes. BBE cells treated with Gn 1 hour prior to serum stimulation exhibited a statistically significant decrease in proliferation as compared to cells only stimulated with serum (P value = 0.0056). BBE cells stimulated with Gn prior to serum stimulation exhibited a marked decrease in pRb expression. While PKC- α inhibition did not affect proliferation with respect to Gn stimulation, we noted that pretreatment of BBE cells with Gn blocked serum-induced nuclear ERK activity and accumulation of nuclear cyclin D1. **CONCLUSIONS:** The ineffectiveness of IEC-18 as a model for this signaling pathway may indicate a role for the GC-C pathway only in times of overexpression, such as cancer. The Gn-stimulated CaCo-2 BBE decrease in proliferation supports this. While

the role of PKC- α in the antiproliferative effects of this pathway are unlikely, the ability of GC-C signaling to block mitogen activated protein kinase activity and subsequent effects on cell cycle proteins provides an avenue for further study.

Testing Multiple Stool Samples Increases the Detection of Intestinal Parasites in Internationally Adopted Children

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Background: The American Academy of Pediatrics recommends screening internationally adopted children (IAC) for intestinal parasites within a few weeks of their arrival to the United States. The literature is unclear as to how many stool specimens should be obtained for optimal recovery of intestinal parasites. At the International Adoption Center at Cincinnati Children's Hospital Medical Center, families are asked to submit 3 stool specimens, collected 24-36 hours apart. Some institutions only test a single specimen in order to decrease costs. In some studies, this approach has yielded sufficiently high sensitivity, however the sensitivity of obtaining a single specimen for intestinal parasite screening in internationally adopted children has not been reported.

Objectives: To determine the overall prevalence of intestinal parasites in internationally adopted children and to determine whether testing multiple stool samples significantly improves detection of intestinal parasites.

Design/Methods: We performed a retrospective review of all ova and parasite tests from children seen at the International Adoption Center between November 1999 and May 2006. We analyzed the distribution of diagnoses according to age, birth country and institution status. For children who submitted more than one specimen, we compared diagnostic results between tests to determine differences in detection rates.

Results: Overall, 891 IAC from 33 countries were available for analysis. Overall 27% of children had a pathogenic intestinal parasite. *Giardia lamblia* was most frequently recovered (18%) followed by *Blastocystis hominis* (10%) and *Dientamoeba fragilis* (6%). In the analysis of children with three stool specimens submitted (n=672), in those with *Giardia lamblia*, only 58% would have been identified with

a single stool specimen. A second and third stool sample identified 11% and 30%, respectively. Similar findings were seen with other pathogens.

Conclusions: Internationally adopted children are at high risk for intestinal parasites. Screening with three ova and parasite tests significantly increases the identification of the pathogens most commonly seen. Three ova and parasite tests should be recommended for all internationally adopted children for routine screening upon arrival to the United States.

***Hlx* regulation of smooth muscle/mesenchymal promoters**

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Background and Significance: Intestinal peristalsis is mediated by enteric smooth muscle that is derived from primitive intestinal mesenchyme. However, very little is known about enteric smooth muscle differentiation. *Hlx* is a homeobox transcription factor gene that is expressed in embryonic mouse intestinal mesenchyme and is required for growth of the intestine. Previous work has shown that *Hlx* is required for normal smooth muscle differentiation.

Hypothesis: *Hlx* regulates expression of enteric smooth muscle in the GI tract.

Methods: Computational biology resources (MultiPipmaker, TraFAC, Genome TraFAC, BLAST) were used to identify conserved regions and regulatory sites. These highly conserved gene sequences were used to direct preparation of deletion constructs (in luciferase reporter vectors) for assay of baseline promoter activity and regulation by *Hlx*. A truncation mutant of *Hlx* was also prepared by point mutation. Promoter constructs and *Hlx* expression constructs were transfected in *Hlx* knockout cell lines in triplicate, and luciferase activity was measured.

Results: We found that the enteric smooth muscle genes are highly conserved, including elements that have been previously shown by others to be important for gene regulation. These results have been used to plan the preparation of deletion constructs to identify more precisely the regions required for *Hlx* regulation of gene expression; the preparation and testing of these constructs is in progress. Truncation of the *Hlx* coding region resulted in a loss of gene regulatory activity. It remains to be determined whether this is due to the synthesis of an unstable or an inactive protein.

Conclusion: *Hlx* regulation of enteric smooth muscle gene expression is likely mediated through conserved regions of enteric smooth muscle promoters. Understanding how *Hlx* regulates gene expression will

provide insights into the mechanisms underlying enteric smooth muscle differentiation.

Application of MATLAB Digital Image Analysis to Measure Pharyngeal Wall Compliance in Obese Adolescents with Sleep Apnea

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Background: Obesity is a major risk factor for pediatric obstructive sleep apnea (OSA); however, the exact pathophysiologic mechanism remains unclear. The purpose of this study was to measure pharyngeal wall compliance in obese adolescents using MATLAB program analysis of cine MR imaging and to compare pharyngeal wall compliance between adolescents with OSA and those without OSA.

Methods: A MATLAB program developed by the Department of Aerospace Engineering at the University of Cincinnati was used to analyze the cine MR images. The images were taken in the identical axial plane at the mid-tongue level, corresponding to the base of the C2 vertebra. Images were taken once per second throughout the respiratory cycle, and 128 images total were taken and analyzed per subject. The MATLAB program analyzed the images by first determining an airway boundary and then using this determination to directly calculate the airway cross-sectional area. For each subject, the ten maximum cross-sectional areas and the ten minimum areas were averaged to determine the upper and lower limits of the airway range, respectively. Pharyngeal compliance was then defined as the normalized range, or the range divided by the average cross-sectional area of all 128 images. The OSA and control groups were then compared using unpaired student's t test or Wilcoxon test for continuous variables and Fischer's exact test for categorical variables.

Results and Conclusions: The imaged control group (n=11) did not differ from the OSA group (n=10) in their age or gender. The normalized range of the airway cross-sectional areas in the control group was 0.49 (± 0.15) compared to the OSA group normalized range of 0.68 (± 0.17) ($p=0.014$), leading us to conclude that a significant difference exists in the wall compliance between the groups. This also

led us to conclude that increased pharyngeal compliance likely plays an important role in the pathogenesis of OSA among obese adolescents.

Differences In Milk Adiponectin Concentration Between Women with and without Gestational Diabetes.

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Background: While it is clear that prenatal exposure to gestational diabetes has negative health consequences, recent studies suggest that the offspring of gestational diabetic mothers who are breast-fed have a poorer metabolic prognosis. Thus, identification of factors that differ in the milk from gestational diabetic mothers may provide clues to novel therapeutic strategies to reduce later obesity. Adiponectin is a likely protective candidate because it is associated with lower adiposity and favorable metabolic profiles including reduced risk of gestational diabetes. Further, adiponectin is present in human milk. Therefore, the purpose this project was to compare the concentrations of milk adiponectin in gestational diabetic mothers to healthy control mothers.

Methods: To accomplish this goal, we recruited mothers with gestational diabetes from the Cincinnati area. Height and weight measurements and milk and serum samples were collected during a home visit at approximately 1 week postpartum. Milk adiponectin concentration was obtained using radioimmunoassay on skimmed milk at 1:3 dilution. 16 control milk samples were used for comparison from the Research Human Milk Bank (RHMB).

Results: Four subjects were enrolled from June through September. Two milk samples from gestational diabetic mothers were obtained from RHMB. Adiponectin levels were 45.2 ± 12.1 versus 41.7 ± 11.8 ng/mL for GD versus control mothers. This difference was not statistically different ($p = 0.55$). Further, BMI did not appear to be related to milk adiponectin concentration ($p > 0.50$).

Conclusion: Milk adiponectin concentration in gestational diabetic mothers was not lower than the control group. Given this small sample size, additional samples will be required to confirm these findings. However, if milk adiponectin truly does not differ between the two groups, proteomics analyses will be used to identify other candidate proteins with differences.

Use of the Stunkard figural stimuli scale to predict risk of diabetes and early breast development.

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Background: The Stunkard Figural Stimuli were originally developed as a psychological tool for body image assessment. In 2001, CM Bulik established BMI norms for each silhouette in a population-based study (n=16, 728 females). Stunkard figures could provide a tool to characterize family history of obesity (FOB), useful in risk prediction for conditions such as Diabetes Mellitus Type II (DMII) or early breast development (which increases risk of breast cancer).

Specific Aims: To validate that a self-selected Stunkard figure correlates well with the BMI calculated from height and weight and the observer-selected Stunkard figure; to determine if the known associations between 1) high BMI and family history of Diabetes Mellitus II; 2) higher BMI in young girls and higher BMI in adult female relatives; and 3) timing of breast development onset and BMI; can be detected when Stunkard figures are used;

Methods: Data were collected via questionnaire from campus women (height, weight, and Stunkard figure for self) and Growing Up Female mothers (height, weight and Stunkard figure for self, mother [M] and maternal grandmother [MGM]). For the validation study (N=32), the respondent also had a Stunkard figure selected for them by observer. For the other analyses, data of GUF mothers was used in analyses with their family medical history and daughter's Tanner breast stage at the second year exam.

Results: In validation, agreement between calculated BMI and both self-selected and observer-selected Stunkard was excellent ($p < 0.0001$). For persons with $BMI \geq 30$ (obese) both self-selected and observer-selected Stunkard figures were about one level lower than that represented by the person's calculated BMI (-4.4 kg/m² for self-selected, -3.54 kg/m² for observer selected).

When Stunkard figures are used to characterize BMI in either mother [M] or both mother and maternal grandmother [MGM], FOB is a good

predictor of family history of DM II (OR= 1.15 and 1.34) but did not predict timing of breast development (OR=0.93).

Conclusions and Significance: Findings of this pilot study support the need for a larger population study to explore the efficacy of use of Stunkard figures as a measure of family history of obesity, with actual measurements of maternal and paternal grandmothers' BMI.

Early Prediction of Acute Renal Injury Following Cardiac Catheterization

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Background: Contrast agents are well known nephrotoxins, and the incidence of acute renal failure (ARF) is estimated to be 15% of all patients following cardiac catheterization. In clinical settings, ARF is typically diagnosed by measuring serum creatinine. This is an unreliable indicator during ARF. The lack of early biomarkers for ARF in humans has crippled our ability to effectively treat patients in a timely manner. Recent studies have identified neutrophil gelatinase-associated lipocalin (NGAL) as a novel predictive biomarker of ARF following cardiopulmonary bypass surgery.

Apart from elevated levels of urinary biomarker after contrast administration, there is a possibility that protective proteins in the kidney might help renal tubule cells escape injury, which could be identified before catheterization. Identification of these protective proteins may have immense value not only for risk stratification prior to contrast administration, but also for identification of novel therapies.

Hypothesis and aims: (a) Patients who undergo cardiac catheterization and contrast administration will have elevated levels of a panel of urinary biomarkers, including NGAL, and (b) There exist protective proteins in the kidney that may prevent ARF after contrast administration. Identification and characterization of these novel biomarker proteins is anticipated to provide a more accurate and precise way to identify the early onset of ARF.

Methods: Urine and serum samples were analyzed for NGAL using a validated ELISA technique. Urinary Proteomic Profiling was performed using Surface-Enhanced Laser Desorption-Ionization (SELDI-TOF) Mass Spectroscopy.

Results: All patients who subsequently developed ARF had increased levels of urine and serum NGAL, detected by ELISA within 2 hours of the procedure.

Apart from elevated levels of urinary biomarkers after contrast administration

7 out of 8 examined by proteomic screening who did NOT develop ARF (control patients) had elevated levels of an as-yet unidentified protective protein (4500 Da).

Conclusion: Concentrations of NGAL in the serum and urine are novel, predictive biomarkers of ARF following contrast administration. The presence of a 4500 Da protein in the urine predicts protection from ARF following contrast administration.

THE EFFECT OF SURGICAL APPROACH ON GAIT DURING TOTAL HIP ARTHROPLASTY

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BACKGROUND: Total Hip Arthroplasty (THA) has had significant clinical success by providing patients an overall improved quality of life, physical function, and relief of pain. But despite the relatively high success rates, gait characteristics of individuals may not return to normal for several years after surgery. Few studies have been conducted to examine the effects that antero-lateral (A-L) versus postero-lateral (P-L) surgical approaches may have on post-operative gait dynamics. Researchers hypothesized that an A-L surgical approach for THA would result in less altered gait kinematics after surgery than a P-L approach for the same procedure. **METHODS:** Data was collected for 19 controls and 1 experimental subject during simple pacing tasks. The joints of patients were marked with small reflective spheres, and three dimensional joint position data was collected using a six-camera infrared recording system. Ground reaction forces were also obtained using a force plate apparatus. **RESULTS:** Three dimensional hip and knee angles were calculated based on the position of the reflective markers at heel strike with the following results: Left Hip: CAvg 127.0°, CStD 3.0° (n=4), EAvG 124.2°, EStD 5.6° (n=3); Right Hip CAvg 93.6°, CStD 1.0° (n=6), EAvG 96.2°, EStD 0.0° (n=1); Left Knee: CAvg 175.4°, CStD 1.5° (n=4), EAvG 173.7°, EStD 0.8° (n=3); Right Knee: CAvg 167.0°, CStD 3.6° (n=6), EAvG 166.1°, EStD 0.0° (n=1); Average difference in COM coordinates (x, y, z) between control and experimental: Left Leg (0.000m, -0.021m, -0.040m); Right Leg (-0.025m, -0.006m, -0.038m). **CONCLUSIONS:** Preliminary results suggest that calculated values for hip and knee angles show no difference between the control and experimental group. Data from COM calculations show differences between the groups, but further testing will be required to confirm this claim. No support for the hypothesis can be shown without further testing.

The Effect of Warfarin Anticoagulation and INR Intensity upon Hematoma Volume in Patients with Intracerebral Hemorrhage

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Introduction: Intracerebral hemorrhage (ICH) is estimated to occur in 67,000 Americans annually. Among patients with ICH warfarin use prior to onset leads to greater mortality. We sought to determine whether warfarin use is associated with larger hematoma size, which might explain this excess mortality. **Methods:** We identified all patients hospitalized with ICH in the five-county Greater Cincinnati area from 1/05-12/05 by reviewing ICD-9 codes 430-436 at all regional hospitals. Hematoma volumes were measured using the first available CT or MRI scan. Volumes were log-transformed for normality. A generalized linear model was constructed to determine whether international normalized ratio (INR) influenced ICH volume after adjusting for other factors such as patient age, race, and hematoma location. In this model INR level was stratified as < 2, 2-3, and > 3. **Results:** There were 263 patients with ICH, 52 of who were taking warfarin. In univariate comparison, patients on warfarin had a greater mean ICH volume than patients not taking warfarin (22.6 cc (4.9) vs. 12.2 cc (6.1), $p=0.025$). Results of the model are shown below.

	Estimate	Antilog [†]	Lower CI	Upper CI	p-value
Intercept*	2.75	15.61	11.52	21.14	<0.001
INR 2-3	-0.37	0.69	0.34	1.39	0.304
INR > 3	1.02	2.77	1.23	6.23	0.014
Lobar location	0.54	1.72	1.09	2.70	0.020
Brainstem location	-1.80	0.16	0.08	0.35	<0.001
Cerebellar location	-0.07	0.94	0.46	1.89	0.854
Onset to scan [^]	-0.01	0.99	0.98	0.99	<0.001

*Deep cerebral location with INR < 2.

[†]To approximate volume in cubic centimeters, multiply the intercept antilog by the antilog of interest.

[^] For each hour from onset to first CT or MRI scan.

Conclusions: Warfarin use is associated with larger initial ICH volumes. However, this effect appears to occur only for INR values >3 . Larger ICH volume among patients using warfarin likely accounts for part of the excess mortality among these patients.

Evaluating Current Treatment Practices for Acute Adolescent Menorrhagia

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Background Menorrhagia is a clinical condition where menstrual blood loss is greater than 80mL/month, and in severe menorrhagia, hemoglobin levels fall below 10mg/100mLs. Up to 20% of women during their reproductive years may experience excessive blood loss, but the risk is highest during adolescence at the onset of menarche. Between 1964 and 1999, 11 different treatment regimens were published, but none studied adolescent menorrhagia. Also, most recommendations were based on expert opinion and not clinical studies: the only randomized clinical trial studied IV estrogen versus placebo. Because the optimal treatment is still unknown, the purpose of this study is to evaluate current treatment practices in order to better design a clinical practice guideline.

Objective To determine frequency of IV estrogen treatment in:

- 1) a clinical vignette survey of adolescent providers
- 2) a retrospective chart review of adolescents admitted to CCHMC.

Hypothesis 1 More providers report using IV estrogen than oral estrogen.

Hypothesis 2 In hospital admissions, IV estrogen was used more than oral estrogen.

Methods Part I.) A survey describing a clinical vignette followed by a series of questions was administered to attendants at the 2006 North American Society of Pediatric and Adolescent Gynecologists (NASPAG) Annual Clinical Meeting and to the Division of Adolescent Medicine at CCHMC. The survey questions included options for labs, initial treatment and maintenance therapy. Analysis involved descriptive statistics looking at the frequency/percentages of each medical treatment plan, as well as a Chi-squared test for differences in treatment by specialty of the respondent. Part II.) A chart review was conducted of CCHMC admissions from 2000-2005 with admit or

discharge ICD-9 codes 626.2/626.8 who were treated for acute menorrhagia. The analysis was similar to the survey analysis, including descriptive statistics of the patient population and frequency/percentages for treatment plans.

Results Part I.) Fifty-five (n=55) surveys were collected from NASPAG and CCHMC. The respondents included 24 physicians trained in Obstetrics/Gynecology, 19 in Adolescent Medicine, 2 in Pediatrics and 3 in other specialties, with 7 missing data. Thirty of the 55 providers (55%) chose oral contraceptive pills (OCPs) in their initial treatment. Twenty-five (43%) chose a treatment plan that included estrogen. Of that group, 80% (20/25) reported using IV estrogen. Part II.) Twenty-six (n=26) CCHMC admissions from 2000-2005 were included in this study (ages 10-19, mean age 14, average hemoglobin 8.06). Of the 26 admissions, OCPs only were prescribed for 20 (76%) patients, with 7 (27%) admissions given both estrogen and OCPs. Twelve patients (46%) were given estrogen only treatment with 83% (10/12) administered by IV.

Conclusion Although the only clinical trial involving menorrhagia treatment studied IV estrogen, the provider survey of NASPAG and CCHMC adolescent medicine practitioners and the retrospective chart review of CCHMC admissions both indicate that current opinion and actual practice favor oral contraceptive treatment more than oral or IV estrogen only therapy. Further analysis will investigate how training/specialty, transfusion, or admission hemoglobin levels affect which treatment plans are implemented or reported. The results of this evaluation of current treatment practices can be used to design a clinical practice guideline for acute adolescent menorrhagia.

***Bmp4* Gene Expression Analysis in the Murine Inner Ear Using LCM**

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Bmp4 (bone morphogenic protein 4) is a member of the TGF- β family that has been demonstrated to play a role in the regulation of inner ear development. There are three different *Bmp4* transcripts, 1A, 1B and intron 2. Varied transcript expression of *Bmp4* has been reported for

the inner ear and other organs in the mouse model. We hypothesize that at least two of the known isoforms of *Bmp4* in the inner ear are tissue specific (epithelial vs. mesenchymal). LCM (Laser Capture Microdissection) was used to procure cells from inner ear cryosections of CD1 E10.5 mouse embryos that were fixed in 70% ethanol.

The otocyst epithelial and mesenchymal cells expressing *Bmp4* were identified using *In-situ* hybridization on corresponding inner ear cryosections of mice from the same litter that were fixed in 4% Paraformaldehyde. *Bmp4* isoform expression was determined by RT-PCR analysis of RNA obtained from LCM. At E10.5, only transcript 1A not the 1B transcript was detected, in both the epithelial and mesenchymal tissue. By studying the tissue expression of *Bmp4* isoforms in the inner ear at different embryonic stages, the molecules involved in inner ear development can be elucidated. Further studies are currently underway to delineate the *Bmp4* regulation in inner ear development at different embryonic stages.

Effects of a Human Inhibitor-1 Polymorphism (G147D) on Calcium Homeostasis and Contractility In Adult Rat Cardiomyocytes

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The sarcoplasmic reticulum (SR) serves as a source and sink for Ca^{2+} during contraction and relaxation of the heart, respectively. Disruption of this process plays a critical role in inducing heart failure. Phosphorylation of the ryanodine receptor (RyR), a Ca^{2+} release channel of the SR, by protein kinase A (PKA) is believed to be integral to calcium cycling within the cardiomyocyte. Protein Phosphatase-1 (PP1) reverses the effects of PKA through dephosphorylation of RyR. However, PP1 is inhibited by endogenous Inhibitor-1 (I-1). The aim is to determine whether a human polymorphism of I-1, G147D, which has been known to disturb Ca-cycling and contractility, affects phosphorylation of RyR. Our hypothesis is that this specific polymorphism interferes with cardiac function through the altered phosphorylation of RyR. Isolated myocytes from adult rat hearts were infected with adenoviruses containing the G147D mutant I-1, or wild type I-1, or empty vector. Subsequently, basal and phosphorylated states of RyRs were determined by quantitative western blotting. Preliminary results showed that in the presence of isoproterenol stimulation, which activates the PKA pathway, the phosphorylation of RyR was decreased in the G147D mutant as compared to wild type I-1. This indicates that the mutant I-1 may affect cardiomyocyte calcium cycling via reduced phosphorylation of RyR, resulting in altered heart function.