INTERLEUKIN-7 (IL7) AND IL7 RECEPTOR ARE NOVEL MODULATORS IN THE DEVELOPMENT OF PULMONARY HYPERTENSION IN HUMANS AND MICE


Abstract Body:

Rationale: Interleukin 7 (IL7) stimulates alveolar epithelial cell proliferation and higher levels of IL7/IL7R are associated with decreased survival in lung cancer. We reason that ligation of IL7 to IL7R enhances pulmonary artery smooth muscle cell (PASMC) proliferation by increasing cytosolic calcium concentration ([Ca2+]cyto), which regulates pulmonary vasoconstriction and vascular remodeling.

Methods: IL7 levels from plasma samples (96 PAH patients and 46 controls) were measured using IL7 Bio-plexTM Pro assay (Bio-rad). Lung tissues from three non-PAH controls and three PAH patients and a mouse model of hypoxia-mediated pulmonary hypertension (HPH) were used to study IL7/IL7R mRNA and protein levels by Western blotting and real-time PCR, respectively. IL7R-deficient mice (IL7R−/−) and C57Bl6 wild-type littermates (WT) were exposed to normoxia or 10% FiO2 for four weeks (n=11 per group). Right ventricular systolic pressure (RVSP) was determined with a Millar pressure transducer catheter. The right ventricle: left ventricle + septum (RV/LV+S) ratio was calculated. Pulmonary artery remodeling was assessed using Aperio image software. In a cell culture model, human pulmonary arterial smooth muscle cells (PASMC) were stimulated with recombinant IL7 (10 ng/ml) for 6 and 48 hrs.

Results: Plasma IL7, lung IL7/IL7R mRNA and protein levels were significantly elevated in PAH patients and in mouse HPH. Under normoxia, RVSP and RV/LV+S did not differ between IL7R−/− and WT mice. After four-weeks of hypoxic exposure, IL7R−/− mice developed less PH (RVSP 29.19 ± 1.06 vs. 37.28±1.218 mmHg, p<0.0001) and right ventricular hypertrophy (RVH) (RV/LV+S 0.2393±0.0129 vs. 0.4543 ± 0.03210, p<0.003), when compared to WT mice. Hypoxia-induced vascular remodeling was attenuated in IL7R−/− mice (External wall area-internal wall area/external wall area: 0.6218±0.1200 vs. 0.3662 ± 0.0869 [WT vs IL7R−/−, p=0.001]). In normal PASMCs, 6 hrs and 48 hrs treatment with recombinant IL7 enhanced SOCE. BrDu assay demonstrated that IL7 promotes PASMC proliferation in a dose-dependent manner (p<0.01).

Conclusion: IL7 is upregulated in human and experimental PAH. IL7R deficiency protects mice against the development of chronic hypoxia-mediated pulmonary hypertension. These preliminary data suggest that IL7/IL7R contribute to the development of PAH and may be a potential therapeutic target in PH pathobiology.

ABSTRACT FINAL ID: 8

CARDIAC POWER OUTPUT: A COMPARISON OF PULMONARY ARTERY CATHERETER AND TRANSTHORACIC ECHOCARDIOGRAPHIC ASSESSMENT AND ANALYSIS OF TREND IN CRITICALLY ILL SURGICAL PATIENTS

M. Mehmoond, K. Tchorsz, R. Markert, M. Chandra, M. McCarthy. Cardiovascular Disease, Wright State University, Dayton, OH

Abstract Body:

Introduction: Cardiac power output (CPO) (W) is a novel hemodynamic end point of resuscitation. A paucity of data exists as to the non-invasive estimation of CPO in surgical intensive care unit (ICU) patients.

Methods: This 48-hr study enrolled 32 critically ill and/or injured adult surgical patients within 6 hours of ICU admission at a Level I Trauma Center. All patients required mechanical ventilation. Serial pulmonary artery catheter (PAC) and transthoracic echocardiography (TTE) measurements were obtained every 12 hrs (total = 5 points/patient). Cardiac power output (mean arterial pressure x cardiac output)/451 was calculated using cardiac output obtained invasively by PAC and non-invasively by TTE. Pearson correlation coefficient and intraclass correlation (ICC) assessed relationship and agreement, respectively, between PAC and TTE measures of CPO. Analysis of variance compared trends of CPO amongst survivors and non-survivors.

Results: The mean age was 49±20 years, 69% were male, and 84% were PAH patients with a mean Injury Severity Score of 24±10. Cardiac power output from PAC and TTE was significantly related (Pearson correlations, 0.65 to 0.78) and agreed moderately (ICC, 0.65 to 0.76) (all p<0.001). Serial CPO by PAC ranged from (0.66 to 2.7) W; (0.65 to 2.89) W; (0.58 to 2.84) W; (0.6 to 3.03) W; and (0.79 to 2.51) W. There was no difference in trends of CPO in survivors versus non-survivors.

Conclusions: There was moderate to strong correlation and agreement between PAC and TTE derived CPO. None had a very low CPO (≤0.53 W) in this cohort and there was no significant difference in trends of CPO amongst survivors and non-survivors over 48 hours of resuscitation.

ABSTRACT FINAL ID: 32

A MECHANISM FOR RESCUING REDUCED CARDIAC Na+ CURRENT IN HEART FAILURE


Abstract Body:

Background: In heart failure, cardiac Na+ current (INa) is downregulated with a concomitant increase in the Na+/Ca2+ exchanger (NCX). We hypothesized that cAMP activates PKA, decreases NCX activity, and rescues reduced INa.

Methods: Freshly isolated mouse cardiac myocytes were stimulated with forskolin, a PKA activator, and the Na+/Ca2+ exchanger inhibitor, Ni2+. NCX activity was measured using extracellular Ca2+ and fura-2 fluorescence. Ventricular myocytes were transfected with a luciferase reporter construct to measure forskolin-induced NCX inhibition.

Results: Forskolin activated PKA (cAMP accumulation, p<0.001) and decreased NCX activity in mouse cardiac myocytes. NCX activity was decreased by 75% (p<0.05) and, consistent with this, NCX inhibition increased by 50% (p<0.05) in forskolin treated myocytes.

Conclusion: forskolin activates PKA, decreases NCX activity, and rescues reduced INa.

ABSTRACT FINAL ID: 16

CARDIAC POWER OUTPUT: A COMPARISON OF PULMONARY ARTERY CATHERETER AND TRANSTHORACIC ECHOCARDIOGRAPHIC ASSESSMENT AND ANALYSIS OF TREND IN CRITICALLY ILL SURGICAL PATIENTS

M. Mehmoond, K. Tchorsz, R. Markert, M. Chandra, M. McCarthy. Cardiovascular Disease, Wright State University, Dayton, OH

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Methods: This 48-hr study enrolled 32 critically ill and/or injured adult surgical patients within 6 hours of ICU admission at a Level I Trauma Center. All patients required mechanical ventilation. Serial pulmonary artery catheter (PAC) and transthoracic echocardiography (TTE) measurements were obtained every 12 hrs (total = 5 points/patient). Cardiac power output (mean arterial pressure x cardiac output)/451 was calculated using cardiac output obtained invasively by PAC and non-invasively by TTE. Pearson correlation coefficient and intraclass correlation (ICC) assessed relationship and agreement, respectively, between PAC and TTE measures of CPO. Analysis of variance compared trends of CPO amongst survivors and non-survivors.

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Conclusions: There was moderate to strong correlation and agreement between PAC and TTE derived CPO. None had a very low CPO (≤0.53 W) in this cohort and there was no significant difference in trends of CPO amongst survivors and non-survivors over 48 hours of resuscitation.
measurements showed that cADPR and forskolin suppressed mitoROS overproduction induced by elevation of NADH or antimycin A. PKA activation was required for channel upregulation, even when mitoROS production was inhibited with a mitochondrial antioxidant.

Conclusions: NAD+ acted predominately through CD38, which is known to transduce NAD+ signals by generating cADPR. PKA activation was required to raise INa. It appears that both direct modification of the channel and reduction in mitoROS are required to restore INa.

ABSTRACT FINAL ID: 40
TRANSIENT RECEPTOR POTENTIAL VANILLOID 2 MEDIATES MYOCYTE CONTRACTILITY

Abstract Body: Background: We have previously described the Transient Receptor Potential Vanilloid 2 (TRPV2) channel in murine cardiac tissue and have also discovered that its agonism results in a positive inotropic response. Though the mechanism of action of the receptor and the relationship between increased inotropic and vascular changes has not been studied, hence, we performed a series of in vivo and ex vivo experiments on wild type (WT), TRPV2-/- mice and isolated cardiomyocytes from both strains in order to elucidate the mechanism of action of TRPV2 and its association with vascular responses.

Methods: We performed invasive and non-invasive (echocardiographic) assessment of cardiac and vascular function in WT and TRPV2-/- mice under baseline conditions. We also isolated cardiomyocytes from WT and TRPV2-/- mice and measured contractility, SR Ca2+ load and Ca2+ transients. Aortas were dissected, and in some, the endothelium removed mechanically. Analyses of contractile properties of vascular smooth muscle were performed in both intact (+E) and endothelium-denuded (-E) thoracic aorta using a DTM myograph.

Results: Via echocardiography we found that all functional parameters were significantly lower in the TRPV2-/- in comparison to the WT. The ejection fraction (EF), stroke volume (SV) and cardiac output (CO) were all lower in the TRPV2-/- in comparison to the WT littermates by 14.9%, 29.4% and 26.3%, respectively. The baseline indices for contractility were also significantly reduced in TRPV2-/- mice compared to WT as measured via invasive catheterization (dP/dmax: 8480±525 vs 6722±458 mmHg/sec; dP/dt40: 7821±446 vs 5984±334 mmHg/sec). Isolated myocyte data demonstrated higher SR Ca2+ load in the WT mice in comparison to TRPV2-/- (0.65±0.08 vs 0.50±0.04, p=0.14) and significantly higher Ca2+ transients (0.5±0.03 vs. 0.32 ± 0.01 ±0.01). The vascular studies in (-E), showed no differences in developed tension in response to phenylephrine between WT and TRPV2-/- aortas (5.99±0.74 vs 6.07±0.55). In WT and TRPV2-/- vessels with (+E), developed tension in response to phenylephrine was substantially less than in (-E) vessels, but was not different between the two genotypes (1.45±0.12 vs 1.75±0.25 in WT and TRPV2-/-, respectively).

Conclusions: We have demonstrated that TRPV2 abrogation results in decreased myocardial contractility in vivo and in vitro. The effect is due to decreased SR Ca2+ concentration and unlikely to be secondary to vascular differences. The precise mechanism of action of TRPV2 in Ca2+ handling at the myocyte level is currently being studied but remains to be fully elucidated.

ABSTRACT FINAL ID: 44
INTEGRIN β4 PLAYS CENTRAL PART ROLE IN CYCLIC STRETCH-INDUCED ENDOTHELIAL CELL INFILTRATORY RESPONSES
W. Chen, R.K. Sharma, J.G. Garcia, J.R. Jacobson. Medicine, University of IL at Chicago, Chicago, IL

Abstract Body: Rationale: Simvastatin, an HMG-CoA reductase inhibitor, has potent lung vascular-protective effects that are associated with both the marked upregulation of in endothelial cell (EC) integrin β4 as well as decreased agonist-induced integrin β4 tyrosine phosphorylation. Accordingly, we postulate that EC protection by simvastatin is dependent on both of these effects and sought to define further the functional role of integrin β4 as a mediator of EC protection in the context of excessive mechanical stretch at levels relevant to ventilator-induced lung injury (VILI).

Methods/Results: We over expressed wildtype integrin β4 or vector in human pulmonary artery EC prior to challenge with 18% cyclic stretch (CS, 6 h). The media was collected for measurement of inflammatory cytokines. Compared to EC transfected with the vector, over expression of wildtype integrin β4 resulted in a significant increase in CS-induced IL6, IL-8, MCP-1, and RANTES in the media while there was no effect on GM-CSF or VEGF. In contrast, pretreatment of untransfected EC with simvastatin (5 µM, 16 h) significantly inhibited CS-induced increases in inflammatory cytokines. To investigate the importance of specific integrin β4 tyrosine phosphorylation sites on these effects, we transfected various mutant integrin β4 constructs into EC prior to CS (18%, 6 h). Compared to cells over expressing wildtype integrin β4, over expression of a mutant integrin β4 lacking a cytoplasmic tail significantly attenuated CS-induced cytokine expression. In addition, a similar effect was observed in EC overexpressing integrin β4 constructs with specific tyrosines (Y) mutated to phenylalamine (F), including Y1440, Y1526 and Y1640 but not Y1422. Finally, over expression of various integrin β4 constructs with specific deletions including (1) the four fibronectin-like domains, (2) Ca2+-beta domain and (3) the tyrosine activation motif which lies between the second and third fibronectin-like domains also resulted in significantly attenuated CS-induced inflammatory cytokine expression compared to over expression of wildtype integrin β4.

Further, VILI-challenged animals pretreated with simvastatin (20 mg/kg, 16 h) were found to have significantly decreased bronchoalveolar lavage fluid (BAL) cell counts compared to VILI controls (40µL/kg, 4h). VILI-challenged integrin β4 cytosolic tail knockout mice (integrin β4-/-) significantly increased protein concentration and total cell count in BAL comparing to background mice. However, there was no significant difference between VILI challenge integrin β4-/- mice and simvastatin pretreatment integrin β4-/- mice followed by VILI challenge.

ABSTRACT FINAL ID: 58
INTRACARDIAC THROMBUS, MYOCARDIAL INFARCTION, AND FAMILIAL THROMBOPHILIA
J.K. Padda, A. Valdes, C. Richardson-Royer, D. Bowe, C.J. Glueck. Internal medicine, Jewish hospital, Cincinnati, OH

Case Report Body: Background: Intracardiac thrombus after myocardial infarction (MI) is associated with peripheral thrombotic embolization, and the risk for adverse outcomes is amplified by the presence of familial and acquired thrombophilia. We summarize the therapeutic choices in a patient with a large post MI left ventricular (LV) intramural thrombus who was found to have previously undiagnosed familial thrombophilia.

Case Report: A 58 year old Caucasian male, cigarette smoker until age 53, with multi-vehicle coronary artery disease, sustained an anterior MI at age 53 (stents placed in the left anterior descending artery), and a second MI at age 58 (stents placed in the left circumflex artery). An echocardiogram four months after his initial MI revealed persistent LV thrombus, with his LV ejection fraction of 30-35%. At age 58, an echocardiogram revealed a large LV thrombus (1.5 x 1.3 cm) adherent to the akinetic LV apex. He was placed on coumadin, which had to be discontinued after bleeding from an anal fissure. Coagulation tests taken to evaluate the persistent intracardiac thrombus revealed Factor V Leiden heterozygosity and high Factor VIII (236%, UNL <150%). Unable to continue warfarin because of anal fissure bleeding, his cardiologist prescribed Effient (10 mg daily) and Aspirin (81 mg daily).

Family history was pertinent for maternal death at age 68 with vascular disease, and a maternal cousin at age 56 with coronary artery disease. A sister at age 58, one brother at age 60, and one brother at age 50 had diagnosed familial thrombophilia. One brother at age 40 died of an MI, and a sibling at age 60 died of a stroke. One sibling at age 45 had undiagnosed familial thrombophilia.

Discussion: LV thrombi after MI are responsible for significant morbidity and mortality, and 25% of cardiac emboli are associated with acute and chronic MI. Measures of coagulation are not routinely taken in cases with persistent LV thrombi. Factor V Leiden heterozygosity and high Factor VIII are associated with increased risk of venous thrombosis, and arterial
atherothrombosis in cigarette smokers. Familial thrombophilia (Prothrombin G20210A homozygosity) has been associated with spontaneous intracardiac thrombosis without MI and with recurrent intracardiac thrombosis after discontinuation of oral anticoagulation therapy. Intracardiac thrombosis in a patient with cardiomyopathy and protein C and S deficiency have been shown to resolve after 24 days of heparin and aspirin. If LV thrombus is persistent after MI, we suggest an evaluation for familial and acquired thrombophilia, to facilitate anticoagulation and to promote screening of at risk first degree relatives. Once a LV thrombus has formed after acute MI, in a patient found to have familial thrombophilia, the risk of cardiogenic embolism is increased, and, in the absence of contraindications to anticoagulation, or as in this case, bleeding on anticoagulation, consideration should be given to anticoagulation with warfarin or Xarelto. It is not known whether effient and aspirin will be effective in cases like ours, where warfarin and Xarelto could not be safely used.

Abstract Body:
Background: African Americans (AA) with diabetes (DM) are at a disproportionately higher risk of myocardial infarction (MI) and stroke compared to non-African whites with DM. A greater prevalence of subclinical disease (ScD) among those with DM in the AA community may be an explanatory factor. We hypothesized that the prevalence of ScD in AA with DM is high and contributes to cardiovascular disease (CVD) events.

Objective: We assessed and compared the distribution of ScD, CVD event rates and the contribution of ScD to CVD events in those with and without DM in a community based cohort of AA.

Methods: We evaluated 3,276 AA participants [mean age (SD) 53.1(12.4) years, 62.25% women] free of overt CVD who attended Jackson Heart Study (JHS) Exam 1 (between 2000-2004) when ScD assessment was routinely performed (with the exception of CT for coronary calcium that occurred in Exam2). ScD measures included 1) peripheral artery disease (PAD, defined as ankle-brachial index<0.9), 2) high coronary artery calcium (CAC, defined as score>100), 3) left ventricular (LV) hypertrophy (LVH defined as left ventricular mass index>51 g/m2.7), 4) low LV ejection fraction (low EF, defined as an EF<50%), and 5) microalbuminuria (MA, defined as an albumin-to-creatinine ratio>25 μg/mg in men and >35 μg/mg in women). Incident CVD events were defined as an MI, coronary revascularization procedure, or stroke. We compared the distribution of standard CVD risk factors, ScD prevalence and the CVD event risk in those with and without DM (referent). We estimated models adjusting for the presence of ScD and stratifying by the presence versus absence of ScD.

Results: In our study sample 752 (22.9%) had DM. Compared to those without DM, those with DM tended to be older, female, hypertensive, obese, had lower HDL, higher fasting glucose, and higher triglyceride levels. Table 1 compares the distribution of ScD for the two groups and demonstrates the greater odds of CAC, LVH, PAD and MA in participants with DM. Over a mean follow-up period of 6.2 years, the incidence rates of CVD (per 100) were 2.63 in those without DM and 12.23 in those with DM. Table 1 also shows the risk of CVD events by DM and ScD.

Conclusion: In this large community-based sample of AAs, we observed a significantly high prevalence of ScD overall, especially so in participants with DM. Also the presence of ScD conferred an increased risk of CVD in those with DM. Our findings identify ScD as a key mediator of CVD risk in AA overall, including individuals with DM.

Endocrinology/Metabolism
Abstract Final ID: 56

Computationally Informed Identification of Pancreatic Islet Maturation Pathways

Abstract Body:
A major unmet goal of treating patients with diabetes is to maintain blood glucose levels within the physiologic range by providing a renewable supply of insulin that is similar to the endogenous hormone. Recent reports have suggested that insulin-producing cells can be derived from human embryonic stem cells (hESC). However, the protocol used for differentiation has only been able to produce “beta-like” cells, which are not glucose-responsive. We took advantage of previous reports that although insulin-expressing cells appear at approximately E13.5, neonatal mouse islets fail to respond to glucose until after a maturation process, which generally occurs a few weeks postpartum. To gain insights into functional maturation of β-cells in vivo, we performed transcriptomic and proteomic analyses of mouse islets isolated from pups at postpartum day (P) 2, P4, P7, P14, P21, P28, and P35 and compared them with mature islets from 8- and 12-week-old mice. For comparison we used in vitro differentiated Pdx1+ and insulin+ cells from mouse embryonic stem cells that were FACS purified. Integrative bioinformatics analyses revealed global changes in gene expression in islets up to P7 and the in vitro differentiated insulin producing cells resembled islets from pups in the early postpartum stage. Our analyses revealed, for the first time, the dynamics of signaling pathways, metabolic processes, and transcription factors during functional maturation of β-cells.

Conclusion: Our proof-of-concept in vitro stem cell validation assay using PPARY agonists showed that acute short-term modulation of the PPARY signaling pathways up-regulated INS, Pdx1, GCK and Glut2 expression and improved G1S of in vitro differentiated insulin-producing cells. Our study highlights the need for sequential combinatorial treatment with multiple pathway modulators and provides a platform for rational design of a more efficient in vitro maturation protocol for differentiating embryonic stem cells.

Abstract Final ID: 74

Adipose Triacylglycerol Lipase (ATGL) and G0S2 Mediate Effects of FoxO1 on Hepatic Triglyceride Turnover, Lipogenic Gene Expression and VLDL Secretion
W. Zhang, I. O-Sullivan, T. Unterman. Medicine, University of IL and JBVMC, Chicago, IL; S. Bu, M. Mashek, D. Mashek. University of Minnesota, Saint Paul, MN; C. Kahn. Joslin Diabetes Center, Boston, MA

Abstract Body:
FoxO proteins are major targets of insulin action and regulate gluconeogenic, glycolytic and lipogenic gene expression in the liver (JBC 281:10105, 2006). FoxO1 promotes lipid mobilization in adipose tissue through regulation of adipose triacylglycerol lipase (ATGL), and ATGL also regulates lipid turnover in liver. We asked whether FoxO1 also regulates expression of ATGL and its inhibitor, the G0/G1 switch gene protein 2 (G0S2), in liver. Studies in liver-specific transgenic mice show that FoxO1 stimulates ATGL and suppresses G0S2 expression, and studies in FoxO1
knockout mice confirm that endogenous FoxO proteins contribute to regulation of ATGL and G0S2 in liver. Studies in liver-specific insulin receptor knockout (lIRKO) mice, and lIRKO mice in which FoxO1 also was inactivated in the liver indicate that FoxO1 plays an important role in mediating effects of insulin on hepatic expression of ATGL and G0S2 in vivo. Studies utilizing adenoaviral vectors in isolated hepatocytes confirm that FoxO1 stimulates ATGL and suppresses G0S2 expression, and metabolic labeling studies demonstrate that FoxO1 promotes triacylglycerol (TAG) turnover and catabolism through an ATGL-dependent mechanism. Interestingly, suppressing hepatic ATGL expression and/or function in FoxO1 transgenic mice increases hepatic TAG content and also raises circulating TAG levels, reflecting increased lipogenic gene expression and secretion of very low density lipoprotein (VLDL), based on analysis by quantitative PCR, FPLC fractionation of circulating lipoproteins and inhibition of VLDL turnover with Tylazocin. Together, these results demonstrate that FoxO1 regulates hepatic expression of ATGL and its inhibitor, G0S2, and indicate that ATGL plays an important role in mediating effects of FoxO1 on hepatic triacylglycerol turnover, lipogenic gene expression and VLDL secretion.

**ABSTRACT FINAL ID: 80**

**SIMPLE MEASUREMENTS AND FORMULAS FROM ORAL GLUCOSE TOLERANCE TEST PROVIDE ACCURATE EVALUATION OF INSULIN SENSITIVITY, SECRETION AND DISPOSITION INDEX SIMILAR TO THE MORE ELABORATED MODELS: COMPARISON WITH THE MINIMAL MODEL ANALYSIS OF AN INTRAVENOUS GLUCOSE TOLERANCE TEST IN AFRICAN AMERICAN MEN.**

B. Manickam, A. Akbar, E. Barengolts. University of IL at Chicago, Chicago, IL

**Abstract Body:**

**Introduction:** Several indices of insulin sensitivity (SI) and beta cell function derived from oral glucose tolerance test (OGTT) have been proposed and validated against the euglycemic hyperinsulinemic clamp or the intravenous glucose tolerance test (IVGTT). These indices, however, are not sufficiently validated in African American men (AAM) with relatively high burden of chronic diseases.

**Objective:** We aimed at determining which OGTT-derived measurements give the best prediction of the insulin sensitivity (SI), acute insulin release (AIR), and disposition index (DI) values obtained with the minimal model analysis of an IVGTT.

**Methods:** Fifty AAM with a wide range of glucose tolerance randomly underevented OGTT and IVGTT. Correlations between OGTT-derived and IVGTT-derived indices of glyceremia were calculated. The accuracy of the empiric formulas obtained were evaluated with Bland-Altman plots.

**Results:** Mean [SD] values were: age 60 [4.8] years, BMI 32.2 [2.5] kg/m2, IVGTT value (standardized units not included): AIR 449 [291], SI 2.9 [1.14], DI 1159 [798]. Raw correlations of the OGTT-derived indices with the values of IVGTT-derived SI and its reciprocal (1/SI) are shown in Table 1. Surprisingly, our simplistic indices based on peak insulin (Ip) and glucose (Gp), i.e. Insulinogenic Index (IPIp=Ip-Ib/Gp-Gb, where Ib and Gb are basal insulin and glucose, respectively), ratio Ip/Gp, and Ip level give the highest r values without the need of complicated formulas or modeling. Several other simple indices, i.e. ratio of Area under the curve (AUC) to AUC glucose (AUCG) with the formula RAUCG=AUC/AUCG, as well as 1/Cbmin which lm indicates mean insulin value, and AUC of mean insulin (AUCIm) are also rather well correlated to 1/SI. Table 2 shows formulas, r and p values of widely accepted and commonly used indices including Matsuda, Matsu-Ogi and ISSI2. DI and AIR correlation well (r=0.001) with peak glucose (Gp) and AUC of mean glucose (AUCGm) with r values varied between ~0.5 and ~0.45.

**Discussion:** Our data showed for the first time that simple OGTT-derived indices such as r even as well or even better that the indices based on complicated formulas or modeling. The comparison of OGTT-derived and IVGTT-derived indices shows that the physiologic significance of all indices is not similar. The indices derived from OGTT, which predominantly reaveal insulin resistance, correlate better with 1/SI than with SI itself, because insulin resistance is the reciprocal of SI (1/SI = SI).

**Conclusion:** Simple measurements and formulas from OGTT in African American men with high disease burden provide accurate evaluation of IVGTT-derived SI, AIR, and DI similar to the more elaborated models. Further studies in other populations are needed to validate our results.

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**ABSTRACT FINAL ID: 82**

**AAM VETERANS WITH PRE-DIABETES HAVE LOW DIETARY VITAMIN D AND HIGH FAT INTAKE**

T. Chaicha-Brom, I. Ciobotaru, B. Manickam, Y. Eisenberg, V. Arguello, E. Barengolts. Endocrinology, University of IL, Chicago, IL; T. Chaicha-Brom, B. Manickam, A. Akbar, H. Mohiuddin, Y. Eisenberg, E. Barengolts. Endocrinology, Jesse Brown VA Medical Center, Chicago, IL

**Abstract Body:**

**Introduction:** African Americans have a high risk for pre-diabetes and type 2 diabetes (T2DM). The risk factors that enable the progression of pre-diabetes to T2DM are not well delineated in African American male (AAM) veterans who have a high burden of chronic disease. Within this population, increased dietary fat intake and suboptimal vitamin D intake may be risk factors for development of pre-diabetes and T2DM.

**Objective:** To evaluate dietary intake of fat and vitamin D in AAM with pre-diabetes.

**Methods:** AAM were recruited from an inner city Veterans Affairs medical center as part of an ongoing clinical trial that evaluates pre-diabetes risk factors in AAM veterans. Seventy-three subjects were included in the present sample population and they reflect the chronological stage the study is at the moment. Subjects were assigned to either a normal glucose tolerance (NGT) or overall impaired glucose tolerance (IGT). Dietary intake was evaluated by 24-hour dietary recall. Pre-diabetes was defined as A1C 5.7–6.4%. Glucose tolerance was defined based on oral glucose tolerance test. Burden of chronic disease was evaluated by the Charlson comorbidity index. Percentage tissue fat was evaluated by DXA. Dietary data were reported as % of recommended dietary allowance (RDA) for calorie and nutrient intake x 100. Between group comparisons were performed using t-test and Pearson’s correlation. Significant differences were determined for p < 0.05.

**Results:** Among 73 AAM we observed the following [number (%)]: normal glucose tolerance (NGT) - 36 (49%), impaired fasting glucose (IFG) - 14%, impaired GT (IGT) - 8% (11%), combined IFG+IGT 13% (18%), and 6% who had newly diagnosed T2DM. A group that included IFG+IGT and T2DM was defined as advanced impaired glucose tolerance (AIGT). Chronic conditions were highly prevalent: hypertension 68%, hyperlipidemia 54.5%, psychiatric problems including PTSD 72.5%, and cancer 13%. Comparison of NGT vs. AIGT showed no differences in (Mean [SD]): age 57.3 [4.7] vs. 60.2 [4.5] years, BMI 31.7 [2.6] vs. 32.0 [2.9] kg/m2, dietary energy 2338 [626] vs. 2597 [823] kcal, dietary vitamin D 341 [354] vs. 342 [323] IU/day, fat intake 94 [35] vs. 118 [48] % recommended of g/day, and Charlson index 2.03 [1.11] vs. 2.38 [1.14]. There were significant differences (p<0.05 for all) in monounsaturated fat intake 63 [34] vs. 108 [65] % recommended intake g/day, fasting serum glucose 90.4 [4.9] vs. 113.1 [7.9] mg/dl, triglycerides 86.1 [32.8] vs. 136.2 [53.9] mg/dl, and % tissue fat (by DXA) 31.6 [3.7] vs. 36.5 [3.1]. There were positive correlations between dietary vitamin D intake and intake of total calories, total fat, and monounsaturated fat (r=0.27, 0.39 and 0.47, respectively).

**Conclusion:** AAM veterans with pre-diabetes have low dietary vitamin D and high fat intake compared to their own RDAs and to those subjects with...
ABSTRACT FINAL ID: 86

FAT-BONE INTERACTION MAY RESULT IN SUBCLINICAL STEATO-OSTEOMYELITIS AND DECREASED BONE MINERAL CONTENT IN AFRICAN AMERICAN MEN WITH METABOLIC SYNDROME.

V. Arguello. Department of Medicine, University of IL at Chicago-Advocate Christ Medical Center, Chicago, IL. B. Manickam, A. Akbar, H. Mohiuddin, V. Eisenberg, E. Barengolts. Department of Endocrinology, University of IL Medical Center, Chicago, IL.

Abstract Body:

Introduction: Data on the association of the metabolic syndrome (MetS) with bone mineral density (BMD) and femoral fracture risk in men is inconsistent showing either higher or lower fracture risk. In older men with MetS a lower risk of fracture was related to hypertriglyceridemia. Previous studies also suggest that obesity is associated with higher BMD but chronic conditions including T2DM, chronic kidney disease, and depression are associated with increased fracture risk.

Objective: To explore the interaction between femur bone mineral content (BMC) and adiposity in African American men (AAM) with MetS and high burden of chronic disease.

Methods: AAM were recruited from an inner city Veterans Affairs medical center as part of an ongoing clinical trial evaluating pre-diabetes risk factors in AAM veterans. Body composition was evaluated by DXA. We used several parameters to represent femur BMD because body composition did not provide direct measurement of femur BMD. These parameters included “gynoid” BMC and BMC adjusted for total gynoid tissue mass (%-Gyn-BMC = Gyn-BMC/total Gyn tissue x 100) as well as Leg-BMC and Leg-BMC adjusted for total leg tissue (%-Leg-BMC = Leg-BMC/total leg tissue x 100). We used “android” fat to represent amount of the visceral adiposity. Burden of chronic disease was evaluated by Charlson comorbidity index. MetS was defined by AHA/NCEP-ATP III criteria.

Results: Characteristics of the patients included in the study are shown in Table 1. In the entire group chronic conditions were highly prevalent: hypertension 68%, hyperlipidemia 54.5%, psychiatric problems including PTSD 72.5%, and cancer 17%. The Gyn-BMC was higher in MetS+ vs. MetS- but this difference disappeared after adjustment for total gynoid tissue mass (%-Gyn-BMC). Conversely, there was no difference in Leg-BMC between Met- and MetS+ groups but after adjustment for total leg tissue (%-Leg-BMC) Leg-BMC in MetS+ appeared significantly lower than that of MetS- group. There was higher amount of fat in both gynoid and leg regions in MetS+ vs. MetS- group. Correlation analysis of the entire group showed significant (p<0.05) positive association between Gyn-BMC and triglycerides (r = 0.36) and both were predictive of the lower fracture risk in the previous study (Szule P et al. 2010). There also was a negative association between %-Gyn-BMC and Android-Fat (r = -0.42, p<0.05) suggesting that systemic inflammation represented by the android visceral fat may be interacting with bone mineral.

Summary and Conclusion: Our results show decreased femur BMC in AAM with MetS compared to men without MetS and possible interaction between systemic and local inflammation (represented by android and gynoid fat, respectively) and bone mineral. Our data suggests that fat-bone interaction results in variable extent of subclinical steato-osteomyelitis and subsequent reduction of bone mineral. This may in part explain the contradictory results from previous studies on the risk of femoral fractures in patients with MetS.

ABSTRACT FINAL ID: 90

INTERPLAY BETWEEN GENOTYPE, TEMPORAL PHASE, AND AGE IN CIRCADIAN-INDUCED DIABETES MELLITUS AND ß-CELL FAILURE.

M. Perelis, B. Marcheva, Y. Kobayashi, C. O'Mara, J.T. Bass. Endocrinology, Feinberg School of Medicine, Northwestern University, Chicago, IL.

Abstract Body:

The molecular clock is composed of an auto-regulatory transcription-translation feedback loop that maintains alignment between internal biochemical processes and the sleep-wake/feeding cycle. Circadian oscillators are deployed to maintain metabolic constancy throughout the entire 24-h solar cycle but how local tissue clocks exert these effects has been unclear. We have shown that cell autonomous expression of Bmal1 , a HHLH-PAS domain transcription factor, within endocrine pancreas is essential for glucose-stimulated insulin secretion (Marcheva, et al 2010). Yet whether clock function in early development or later life is important in this process has not been understood. Here we show that targeted ablation of either Clock and Bmal1 selectively within the pancreatic islet results in impaired glucose tolerance due to reduced insulin secretion. Cellular analyses of islets lacking either Clock or Bmal1 reveal refractory responses to both cyclase activators and potassium channel agonists, indicating defective ß-cell function at the very last stage of stimulus-secretion coupling. Surprisingly, in isolated wild-type islets, insulin secretagogues exhibit strong circadian variation. Disruption of either Clock or Bmal1, the two components of the forward limb of the clock, resulted in similar metabolic phenotypes, consistent with overlapping molecular targets. Moreover, conditional ablation of the pancreatic clock in mid-adult life was sufficient to induce impaired glucose-stimulated insulin secretion. These results demonstrate a primary role for the islet clock in mammalian glucose homeostasis and reveal intrinsic rhythmic properties in ß-cell function.

ABSTRACT FINAL ID: 96

HIGH PREVALENCE OF GCK- AND HNF1A-MATURITY-ONSET DIABETES OF THE YOUNG (MODY) IN THE US MONOGENIC DIABETES REGISTRY.

D. Carmody, J. Hwang, J. Dickens, G.J. Bell, L.H. Philipson, S.W. Greckey, R.N. Naylor. University of Chicago, Chicago, IL.

Abstract Body:

The established MODY calculators and screening tools are based on data derived from European populations. We sought to assess these MODY screening criteria among patients in the University of Chicago Monogenic Diabetes Registry (http://monogenicdiabetes.uchicago.edu) and investigate the common forms of MODY in the US population.

We compared the phenotypical features of those found to have a causal mutation (MODY +) with those found to have no significant variations (MODY -) within the 3 most common MODY genes. Probands >1yrs with completed HNF1A and HNF4A sequencing plus GCK (if clinically indicated) were considered. Each group was evaluated to identify if patients met at least one of the screening criteria for testing:

a) “Type 1 DM” with either
- Negative antibodies or - Evidence of endogenous insulin 3-5 yrs. post diagnosis

b) “Type 2 DM” with either
- BMI <31 kg/m2 or
- No signs of insulin resistance/metabolic dysfunction
c) “Type 1 or 2 DM” with
- 2 consecutive generations of diabetes or dysglycemia

Results are presented in Table 1. 42.7% of all probands >1yrs. in the registry listed for GCK sequencing were found to have a causal mutation. 18.7% and 3.3% of all >1yrs. listed for HNF1A and HNF4A sequencing respectively, were found to have a causal mutation.

Our data demonstrate the high success rate of diagnosing GCK-MODY and HNF1A-MODY when using strict screening criteria. However, the high percentage of subjects also meeting screening criteria but with negative
testing for GCK, HNF1A and HNF4A highlights the difficulty in identifying patients for MODY testing. The MODY subjects represent an interesting cohort for study of rare or novel causes of MODY.

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**ABSTRACT FINAL ID: 106**

**CHOLECYSTOKININ PROTECTS PANCREATIC BETA CELLS FROM APOPTOSIS**

S. Sirinvaravong, J.A. Lavine, D.B. Davis. Medicine/Endocrinology, University of WI, Madison, WI; M. Baan. University of WI, Madison, WI

**Abstract Body:**

Cholecystokinin (CCK) is a hormone classically associated with digestive function. We have previously shown that CCK is also expressed in the pancreatic β-cell in the setting of obesity. Maintenance of adequate pancreatic β-cell mass is key to the ability to produce adequate insulin and maintain normal blood glucose levels. Loss of CCK in obese mice leads to diabetes with decreased β-cell mass due to increased apoptosis. In vitro, addition of CCK was able to rescue CCK knockout islets from apoptosis due to endoplasmic reticulum (ER)-stress.

To determine if over expression of CCK is sufficient to prevent β-cell death and improve glycemic control, we generated a transgenic mouse with CCK gene expression driven by the mouse insulin promotor (MIP). No differences were seen in glucose and insulin levels in young, lean animals where there are few apoptotic stressors on the β-cell.

As animals age, basal rates of β-cell apoptosis increase and there is a gradual decline in β-cell mass. We saw no change in glucose levels when comparing lean MIP-CCK and wild type mice up to 48 weeks of age; however male mice exhibited a 2-fold increase in insulin levels with age. When we measured β-cell area in the pancreata of animals aged 12 to 15 months, we found that aged MIP-CCK mice had higher fractional β-cell area (5.07±1.64% vs 1.38±0.41%, p <0.05). To examine the effects of MIP-CCK overexpression in a model of induced β-cell apoptosis, we treated 10 to 13 week-old mice with multiple low doses of the β-cell toxin streptozotocin (STZ). We found that MIP-CCK mice are resistant to STZ-induced diabetes, as measured by fasting glucose values (186 ± 51 mg/dl vs. 264 ± 71 mg/dl at 21 days after STZ, p<0.05). In addition, these animals had a 2-fold reduction in the level of β-cell apoptosis after STZ treatment. Next, we wanted to test the ability of CCK to protect from ER-stress mediated apoptosis in vivo. The Akita mutation is a mutation in proinsulin that prevents it from folding properly and ultimately leads to ER stress and β-cell apoptosis. Mice that are heterozygous for the Akita mutation develop significant hyperglycemia by 12-14 weeks of age. However, MIP-CCK, Akita heterozygotes have lower blood glucose levels at 12 weeks, suggesting a protective effect of CCK overexpression.

Finally, we directly tested the ability of CCK to protect from apoptosis due to cytokines in a mouse insulinoma cell line. Treatment of MIN-6 cells with a CCK adenovirus reduced cytokine-mediated apoptosis by 40%.

In summary, our data shows that over expression of CCK protects β-cells from apoptosis and can be beneficial in models of insulin resistance and β-cell death. This is a novel role for CCK in the islet and opens potential avenues for therapeutic development to prevent or treat patients with diabetes.

**ABSTRACT FINAL ID: 42**

**ASSEMBLY AND ANALYSIS OF INVERSION BREAKPOINTS IN DROSOPHILA MELANOGASTER BALANCER CHROMOSOMES**

D.E. Miller, S. Hawley. University of Kansas Medical Center, Kansas City, MO; D.E. Miller, S. Hawley. Stowers Institute for Medical Research, Kansas City, MO

**Abstract Body:**

Multiply inverted chromosomes, known to Drosophila researchers colloquially as balancer chromosomes, are a long-established and important resource in the fly toolkit. For example, as a suppressor of recombination, the balancer allows mutant genes with deleterious phenotypes to be kept in stocks. Similarly rearranged and inverted chromosomes are also found in natural populations as well as in human disease, such as cancer. Here, we describe the assembly and analysis of a multiply inverted chromosome in Drosophila melanogaster, and we describe a workflow that may find clinical applications as whole-genome sequencing becomes prevalent in the improved health of patients.

**ABSTRACT FINAL ID: 102**

**HIV/AIDS DATA BASE ASSISTS IN ANSWERING CLINICALLY RELEVANT QUESTIONS**

P. Gulick, L. Dale. Infectious Disease, Michigan State University, East Lansing, MI; P. Gulick, A. Amalfitano, L. Dale, J. Beck. College of Osteopathic Medicine, Michigan State University, East Lansing, MI; A. Amalfitano, S. Kim, J. Scott. Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI; N. Kaminski. Center for Integrative Toxicology, Michigan State University, East Lansing, MI

**Abstract Body:**

Despite much effort and research surrounding the development of new drugs against HIV, there is limited research that focuses on the impact of drugs on T cell function and the ability for some patients to naturally ward off the disease. Headed by Dr. Peter Gulick and Linda Dale, the objective of the research is to create a confidential, voluntary and de-identified data base in which a large pool of HIV/AIDS patient’s records can be registered. The registry will be created by recruiting patients from Dr. Gulick’s patient population from Michigan. Once the registry is complete, many notable Michigan State University researchers will be looking for ways to improve clinical care of HIV/AIDS patients. These will include the impact of cannabinoids on the immune system, analyzing the genetic factors which allow patients to naturally suppress the virus, and the different functions of natural killer cells. Ultimately we plan to develop an HIV-focused clinical, behavioral basic discovery research program which translates into the improved health of patients.

**ABSTRACT FINAL ID: 66**

**GASTROENTEROLOGY/CLINICAL NUTRITION**

**TRENDS IN PEDIATRIC INFLAMMATORY BOWEL DISEASE, RESULTS FROM A UNITED STATES DATABASE: 2000-2009**

C. Pant, M.P. Anderson, J.E. Grunow, J.A. O’Connor. Pediatric Gastroenterology, OUHSC. Oklahoma City, OK; T.J. Sfera. Pediatric Gastroenterology, Case Western University, Cleveland, OH; A. Deshpande. Infectious Diseases, Case Western University, Cleveland, OH; J.R. Philpott. Gastroenterology, Cleveland Clinic, Cleveland, OH

**Abstract Body:**

Objective: Recent studies suggest an increasing incidence of inflammatory bowel disease (IBD) in children. However, the impact of this increase on the secular trends of inpatient care and disease burden in hospitalized children with IBD is unknown. Therefore, we sought to evaluate for changes in the incidence and burden of pediatric IBD in the United States from 2000 to 2009.

Methods: We used the U.S. Healthcare Cost and Utilization Project Kids’ Inpatient Database. Data were weighted to generate national-level estimates.
Results: We identified 61,779 cases of pediatric IBD during four triennial periods from 2000 to 2009. Between the years 2000 to 2009, the rate of hospitalization of children with any diagnosis of IBD increased from 43.6 to 72.0 (cases per 10,000 total hospitalizations entered into the database per year; P < 0.001). Specifically, between the years 2000 to 2009, for Crohn’s disease (CD) the rate increased from 28.3 to 45.7 (P < 0.001) and for ulcerative colitis (UC) from 15.2 to 26.1 (P < 0.001). There was an increasing trend in the rate of hospitalization in pediatric cases of IBD overall, and CD and UC, individually (P < 0.001) between each year of study (2000, 2003, 2006 and 2009). The age distribution of hospitalized children did not change. Mortality (1 per 1,000 cases of IBD) and length of hospital stay (LOS, 4 days, median) remained constant over the decade. Hospitalization charges (adjusted for inflation) increased (median, $11,614 to $20,724, P < 0.001). Significant increasing trends were found for comorbid disease burden and systemic complications (including electrolyte disturbances and anemia), and the need for blood transfusion and parenteral nutrition (P < 0.001 for each). There, also, was an increase in the number of cases with fistulizing, obstructive, and perianal disease (P < 0.001 for each). In comparison of IBD and non-IBD cases, those with IBD had lower mortality, longer LOS, and higher charges (P < 0.001 for each). Case-control matching demonstrated a lower risk of death (adjusted odds ratio, aOR 0.25, 95% CI, 0.20-0.31), longer LOS (aOR 2.48, 95% CI, 2.40-2.50), and higher charges (aOR 1.92, 95% CI, 1.88-1.96) in those with IBD.

Conclusions: These results demonstrate an increasing trend in the number of pediatric cases admitted to the hospital with IBD. Moreover, there is an increasing trend in disease-specific and systemic complications in these children along with an increase in cost for hospital stay. These findings are consistent with earlier studies demonstrating that the epidemiology of pediatric IBD is changing as demonstrated by an increase in hospitalized cases. Also, these data suggest that there has been an increase in the severity and frequency of complicated disease.

ABSTRACT FINAL ID: 68
COAGULOPATHY IS AN INDEPENDENT PREDICTOR OF IN-HOSPITAL MORTALITY AMONG PATIENTS UNDERGOING TRANSJUGULAR LIVER BIOPSY: A NATIONWIDE INPATIENT SAMPLE STUDY

N.K. Mazumder, Medical University of the Americas, Charleston, Saint Kitts and Nevis; M. Mujib, W.S. Aronow, Westchester Medical Center, Valhalla, NY

Abstract Body:
Background: Transjugular liver biopsy (TJLB) is often the preferred approach among potential liver biopsy candidates with known or suspected coagulopathy. However, the effect of coagulopathy on outcomes of patients undergoing TJLB has never been studied from a national database.

Methods: We used the 2010 Nationwide Inpatient Sample (NIS) to evaluate patients who obtained a TJLB (ICD 9 procedure code 50.13). Sample weights were developed to enable nationwide estimates. Of a total of approximately 8 million hospitalizations in NIS 2010 database, 958 had undergone TJLB in 2010. National estimate (using sample weights) of TJLB was 4,954. Patients were stratified by either the presence or absence of any coagulopathy (Agency for Healthcare Research and Quality comorbidity measure: coagulopathy). Multivariable logistic regression models were used to assess the effect of coagulopathy on in-hospital mortality for patients undergoing TJLB.

Results: Of 4,954 TJLB patients, 1703 (34%) had any coagulopathy. TJLB patients had a mean age of 50 (±16) years, 44% were women and 35% were non-whites. In-hospital mortality occurred in 19% and 6% of patients with and without any coagulopathy respectively (unadjusted odds ratios, 3.47; 95% confidence intervals, 2.88-4.19; P < 0.001). After controlling for patient risk factors, hospital characteristics and operative volume, patients with any coagulopathies independently conferred the higher adjusted odds of in-hospital mortality (adjusted odds ratios, 3.56; 95% confidence intervals, 2.20-5.76; P < 0.001). TJLB patients with any coagulopathy had a longer mean length of stay (mean stay, 18 ± 13 days, P < 0.001) and a higher mean hospital charges ($207,055 vs $124,114, P < 0.001).

Conclusion: In this nation-wide study, coagulopathy was common among TJLB patients and was independently associated with in-hospital mortality. Coagulopathy in TJLB was also associated with longer length of hospital stay and an increased hospital cost. Further prospective studies may be needed to reassess the safety of TJLB among patients with coagulopathy.

GENETIC & MOLECULAR MEDICINE
ABSTRACT FINAL ID: 6
MICRORNA-BASED APPROACH EXPANDS ON GENOME-WIDE ASSOCIATION STUDY AND IDENTIFIES SNPs IMPORTANT FOR RESPONSE TO GLUCOCORTICOIDS IN ASTHMA PATIENTS

R. Huang, H. Im, E.R. Gamazon, D. Lenkala, K. Wu, N.J. Cox, University of Chicago, Chicago, IL; G. Clemmer, S.T. Weiss, K. Tantisira. Channing Laboratory, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA

Abstract Body: Our objective is to take a concerted translational effort to elucidate the genetic etiology of sensitivity to glucocorticoids (GCs), which are some of the most commonly, used agents in treating inflammatory diseases. Our previous work demonstrated that by integrating gene expression into genome-wide association studies (GWASs), one can identify genetic variations that are functionally relevant to the phenotype of interest. Here we expanded this integrative genomic approach to include the use of genome-wide expression of microRNAs (miRNAs), a set of small non-coding RNAs that play an important role in diverse biological processes, and applied this approach to patients from the Childhood Asthma Management Program (CAMP), a randomized trial of asthma treatment. Utilizing a linear mixed effects model, we characterized the longitudinal phenotypic data (repeated measurement of FEV1, an indicator of lung function every 4 months for 4 years) from CAMP The log2-transformed difference between FEV1 over time post budesonide (an inhaled GC) treatment and baseline FEV1 was used as the phenotype of interest. Genome-wide SNP data were obtained from budesonide-treated subjects using Illumina 550K and 610K arrays. We found 12 SNPs that are associated with GC sensitivity at FDR<0.25. The most significantly associated SNP (p=2.8E-8) is also associated with the expression of 8 miRNAs, many of which have been implicated in airway function and inflammatory pathway. This study suggests that genetic variation may influence miRNA expression levels with important phenotypic effect on response to GC in asthma patients.
Abstract Body:

Rationale: Non-muscle myosin light chain kinase isoform (nmMLCK) is centrally involved in driving rearrangement of the cytoskeleton, which regulates vascular endothelial barrier function, angiogenesis, endothelial cell apoptosis, and leukocytic diapedesis. In vivo studies implicated nmMLCK as an attractive target for ameliorating the adverse effects of dysregulated lung inflammation. Deletion or silencing of nmMLCK produced greater protection against acute lung injury (ALI) and ventilator-induced lung injury (VILI) and significantly decreased alveolar and vascular permeability and lung inflammation. However, little is known regarding the role of nmMLCK in the growth of lung tumors and its influence on the prognosis of lung cancers.

Methods: Using an experimental murine model of lung injury induced by mechanical ventilation with increased tidal volume (the VILI model), we characterized the top differentially expressed genes between VILI-challenged wild-type (WT) mice and nmMLCK knockout (KO) mice. These genes formed the basis of a multivariate molecular predictor of overall survival that was trained and validated in lung cancer.

Results: 370 genes were found to be differentially expressed between VILI-challenged WT mice and nmMLCK-KO mice. Among these genes, we only included the genes differentially expressed between VILI-challenged WT mice and WT controls. We also excluded the genes differentially expressed between VILI-challenged nmMLCK-KO mice and WT controls. In total, 45 mouse genes were identified. Gene ontology analysis indicated that ErbB signaling pathway was the top pathway enriched in the 45 mouse genes (P < 10^-3). We matched the 45 mouse genes to their human orthologs, 38 human orthologous genes (38-gene signature), such as ADORA2B, CXCL2, CXCL6, DIO2, and EGFR, were obtained. A risk scoring system was developed based on the expression of the 38 genes. We trained the 38-gene signature in one human lung cancer expression dataset and tested it in several independent cohorts. We found that the risk score based on the 38-gene signature can predict significantly reduced overall survival in lung cancer (P < 10^-2). This signature predicted outcome independently of, but cooperatively with, standard clinical and pathological prognostic factors including patient age, lymph node involvement, tumor size, and tumor grade.

Conclusions: This study provides the first prognostic cancer gene signature derived from a murine model of nmMLCK-associated lung inflammation. Activation of nmMLCK-involved pathways contributes to tumor growth and progression in lung cancers. These findings support the notion that nmMLCK is an attractive candidate molecular target in lung diseases.

HEMATOLOGY/ONCOLOGY

ABSTRACT FINAL ID: 10

SUDDEN SENSORYNEURAL HEARING LOSS AND FAMILIAL THROMBOPHILIA

D. Pereira, L. Harvey, C. Glueck. Internal Medicine Residency Program, The Jewish Hospital, Cincinnati, OH

Case Report Body:

Introduction: Sudden Sensorineural Hearing Loss (SSNHL) can be caused by viral coxchitis, microvascular thrombotic events, cranial nerve VIII neoplasm, familial and acquired thrombophilia, autoimmune diseases, trauma, ototoxic drugs, and very rarely, various other causes such as Multiple Sclerosis, Paget’s disease, Meniere’s disease, and sarcoidosis. Also, many cases remain idiopathic. We summarize a case of SSNHL with Familial Thrombophilia.

Case Report: A 62 year old male, being evaluated for hyperlipidemia and insulin resistance developed sudden hearing loss of the left ear. His medical history was also significant for hypothyroidism, obstructive sleep apnea, hepatitis B infection, benign prostatic hyperplasia and hypovitaminosis D. He had no precedent viral symptoms, trauma or ototoxic medication exposure. He further denied headache, local ear symptoms, prior hearing loss, ocular or other neurologic symptoms and physical examination was unrevealing. He was evaluated with audiometry and a sub-total severe left sensorineural hearing loss was confirmed. MRI head and X-ray of the auditory canal were normal. Further laboratory evaluation revealed several previously unknown thrombophilic disorders including: MTHFR gene mutation (C677T homozgyous) with concomitant elevated homocysteine levels (19.6, normal upper limit = 15 umol/l); high factor VII (175%), upper normal limit = 150%); high Factor XI (179%, normal upper limit = 150%); high anti-cardiolipin IgG antibody (27, normal upper limit = 22g/ml). Factor V Leiden was negative, as were protein C and S studies. His homocysteine level was normalized with a daily oral regimen of 15mg of folic acid, 100mg of vitamin B6, 2000mcg of vitamin B12, and 6g of Cystadane. As expected in micro-vascular SSNHL, the patient did not have recovery of his hearing, but has had no further thrombotic events during his 9 year follow up.

Discussion/Conclusion: After the development of SSNHL it is important to evaluate for familial and acquired thrombophilia in order to prospectively protect against future venous and arterial thrombosis which may result in significant morbidity.

ABSTRACT FINAL ID: 12

TREATABLE HIGH HOMOCYSTEINE IS OFTEN THE SOLE THROMBOPHILIA ASSOCIATED WITH THROMBOSIS

L. Harvey, D. Kichura, C. Riffield, D. Pereira, D. Martinez, S. Sah, P. Wang, C. Glueck. Internal Medicine Residency Program, The Jewish Hospital, Blue Ash, OH

ABSTRACT BODY:

Objective: Assess homocysteine as the sole treatable thrombophilia associated with thrombosis.

Design: Assess 82 patients referred for evaluation of coagulation disorders, where PCR measures of the Factor V Leiden, Prothrombin, and MTHFR C677T and A1298C mutations, 46 of whom also had measures of Factors VIII and XI.

Setting: Outpatient clinical research center.

Patients: 82 patients evaluated for deep venous thrombosis- pulmonary embolus (n=36), central retinal vein occlusion (CRVO, n=26), osteonecrosis of the hip (n=20).

Results: The 82 patients with high (>13.5 umol/l) homocysteine, mean: SD 17.6 ±5.4, included 44 men, 38 women, 75 white, 6 black, 1 other, mean: age 58±14. Years. Of 46 patients having both the three PCR and two serologic measures (Factor VIII and XI), 17 (37%) had high homocysteine as the sole thrombophilia, while 17 (37%) had MTHFR mutations, 4 (9%) PTG, 17 (37%) high Factor VIII, and 5 (11%) high Factor XI. Of 82 patients having the three PCR measures, 53 (65%) had high homocysteine as the sole thrombophilia, while 24 (29%) had MTHFR mutations, 3 (4%) Factor VIII, and 5 (6%) PTG.

In 33 patients the median and 25th-75th percentile pretreatment homocysteine 17 (15-20) fell on treatment with folic acid-B6-B12 (5mcg-100mg-2000 mcg/day) to 10 (7-13) umol/L, p<.0001, and 27 of the 33 (82%) patients’ homocysteine fell to normal, <13.5.

Discussion/Conclusion: Treatable high homocysteine factor found in evaluation of patients with thrombotic events, and, as such, should routinely be measured and treated when found.

ABSTRACT FINAL ID: 20

MULTIFOCAL OSTEONECROSIS AND HEREDITARY THROMBOPHILIA

L. Harvey, D. Pereira, P. Wang, C. Glueck. Internal Medicine Residency Program, The Jewish Hospital, Cincinnati, OH

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<td>PTG</td>
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46 had completed data in MTHFR, Factor V, PTG, Factor VIII and Factor XI

82 patients had completed data in MTHFR, Factor V and PTG

4.07, 13.5, 27.0
Multifocal osteonecrosis (MFON) is rare, devastating, commonly secondary to corticosteroid therapy, but is also associated with thrombophilia, a treatable etiology not often targeted diagnostically. We focused on under-recognized, treatable thrombophilia-hypofibrinolysis and eNOS T786C-stromelysin 6A mutations (reduced nitric oxide production) in the pathogenesis of MFON.

**Results:** Twelve patients had documented MFON in ≥ 2 different locations (hips, knees, shoulders, etc. 62 joints); 5 patients had received corticosteroids, the most common acquired risk factor. However, all 5 also had underlying thrombophilia, 2 had eNOS heterozygosity. Of the 7 patients without corticosteroids, 5 had ≥ 1 thrombophilia, 5 had eNOS heterozygosity, and 4 had stromelysin heterozygosity. The prevalence of the inherited thrombophilias, eons, and stromelysin mutations (Table 1).

Of the 12 patients, 10 with familial thrombophilia were treated therapeutically and 2 without thrombophilia empirically with low molecular weight heparin (1.5 mg/kg body weight [2 divided doses/day]). Previous unrelenting progression of joint failure and progressive involvement of previously unaffected joints was stopped in 6 patients (all with familial thrombophilia), but the disease progressed in the remaining 6 (4 with familial thrombophilia, 2 without familial thrombophilia).

**Discussion/Conclusion:** Familial thrombophilia, and eNOS T786C and stromelysin 6A mutations appear to be common treatable pathologies for MFON, where the untreated natural history is usually inexorable progression. Screening for familial thrombophilia and eNOS-stromelysin mutations facilitates treatment which may arrest the otherwise progressive loss of joint function.

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**ABSTRACT FINAL ID: 26**

**CONSEQUENCES OF FAILING TO INVESTIGATE FOR FAMILIAL OR ACQUIRED THROMBOPHILIA AFTER DEEP VEIN THROMBOSIS: DEEP VEIN THROMBOSIS FOLLOWED BY RETINAL VEIN THROMBOSIS IN FAMILIAL THROMBOPHILIA**

S. Sah, D. Pereira, C. Glueck. Internal Medicine Residency Program, The Jewish Hospital, Cincinnati, OH

**Case Report Body:**

**Introduction:** Our specific aim was to examine the consequences of failing to investigate for familial or acquired thrombophilia after deep vein thrombosis (DVT).

**Case presentation:** A 55 y. o. male with history of well-controlled diabetes mellitus and hypertriglyceridemia had DVT in his leg in the absence of short or long term immobilization, cancer, or other known pathologies for DVT. His DVT was treated with warfarin for three months, resolved, and no further investigation was done. Four years after the DVT, he developed sudden unilateral loss of vision, and was found to have central retinal vein occlusion (CRVO). Family history revealed paternal DVT. Full evaluation for thrombophilia revealed heterozygosity for the Factor V Leiden R506Q mutation and homozygosity for the MTHFR C677T/C677T mutation known treatable pathologies for CRVO. Treatment was started with Aspirin 81mg, folic acid 5mg, Vitamin B6 100mg, Vitamin B12 1000mcg. On therapy, he has had no subsequent thrombotic events and no deterioration of vision. Since this was his second thrombotic event lifelong anticoagulation is being contemplated.

**Discussion/Conclusion:** Often, as in this case, thrombophilic etiologies of initial DVT are not investigated, and patients are treated empirically with warfarin. Often, a full evaluation for thrombophilia is done only when and if a second thrombotic event occurs. We suggest that after a first unexplained thrombotic event, full evaluation for thrombophilia be done to identify underlying treatable pathoetiologies, and to better understand and ideally prevent future thrombotic events.
A 55 year old white male developed progressively severe right knee pain, with osteonecrosis diagnosed by MRI. Past medical history was significant for hypertension and hyperlipidemia, non-contributory to his osteonecrosis. There was no history of high dose, long term corticosteroids, cigarette smoking or alcohol excess. Assessment of thrombophilia and hypofibrinolysis revealed elevated thrombophilic high factor VIII and hypofibrinolytic high lipoprotein(a). Enoxaparin for 3 months resulted in total resolution of his symptoms. However, after 3 years he relapsed with similar pain in his left knee, with osteonecrosis by MRI. He was again treated with Enoxaparin for 3 months with complete resolution of symptoms. He remains asymptomatic at 2 years follow up.

Discussion: In patients with “idiopathic” osteonecrosis of the knee, we recommend assessment of thrombophilia and hypofibrinolysis and discontinuation of exogenous estrogen or testosterone if familial or acquired thrombophilia or hypofibrinolysis is found. In the absence of contraindication to anticoagulation, anticoagulation with 3 or more months with LMWH (1 mg/kg/day in two divided doses) may stop the progression of osteonecrosis, ameliorate pain, and allow normal function. Beyond 3 months, the duration of therapy is uncertain and further studies are required to determine treatment guidelines.

This case illustrates the importance of evaluating thrombophilia and hypofibrinolysis in patients with idiopathic osteonecrosis, since recognition of underlying, pathologic thrombophilia-hypofibrinolysis provides a therapeutic target which may reverse the otherwise progressive loss of knee function.

INFEKTION DISEASE
ABSTRACT FINAL ID: 36

NEMATODE ASPARAGINYL TRNA SYNTHETASE RESOLVES INTESTINAL INFLAMMATION IN MICE WITH TCELL TRANSFER COLITIS.

M.A. Kron, S. Vodanovic Jankovic. Medicine, Medical College of Wisconsin, Milwaukee, WI; A. Metwally, D. Elliott. Internal Medicine, University of Iowa Carver College of Medicine, Iowa city, IA

Abstract Body:
The therapeutic effects of a controlled parasitic nematode infection on the course of inflammatory bowel disease (IBD) have been demonstrated in both animal and human models. However the inability of individual well characterized nematode proteins to recreate these beneficial effects has limited the application of component immunotherapy to human disease. The nematodes that cause chronic human lymphatic filariasis, Brugia malayi and Wuchereria bancrofti, are among the parasites that induce immune suppression. Filarial lymphatic pathology has been shown to involve NFκ B pathway dependent production of vascular endothelial growth factor (VEGF), and stimulation of VEGF expression has also been reported by interleukin 8 (IL8) via NFκ B pathways. Previously we have shown that the filarial asparaginyl-tRNA synthetase (BmAAsnRS) interacts with IL8 receptors using a combination of extracellular loops that differ from those bound by IL8. PURPOSE: To test the hypothesis that recombinant BmAAsnRS (rBmAAsnRS) might induce an anti-inflammatory effect in vivo, we studied the effects of rBmAAsnRS in an established murine colitis model using T-cell transfer mice. METHODS: T cell transfer colitis mice treated intraperitoneally with 100 μg of rBmAAsnRS four times over two weeks, showed resolution of cellular infiltration in the colonic mucosa, along with induction of a CD8+ cellular response. In addition, rBmAAsnRS induced a rise in IL10 production from CD3+, LPS- and CFR-stimulated splenic cells. Human immune dendritic cells that express IL8 receptors are stimulated by both IL8 and rBmAAsnRS, however the effects on gene expression in the NFκ B signal transduction pathway are distinctly different. CONCLUSION: This work demonstrates a novel anti-inflammatory nematode protein - a putative nematode derived chemokine receptor antagonist, supports the Hygiene Hypothesis and supports continued refinement of alternative immunotherapies for treatment of IBD.

ABSTRACT FINAL ID: 48

DEFICIENT REPORTING AND INTERPRETATION OF NON-INFERIORITY RANDOMIZED CLINICAL TRIALS IN HIV PATIENTS: A SYSTEMATIC REVIEW

P. Thota, A. Deshpande, V. Pasupuleti. Infectious Diseases, Case Western Reserve University, Cleveland, OH; J.A. Collins. Internal Medicine, University of Iowa Carver College of Medicine, Iowa City, IA

Abstract Body:
Objectives: Non-inferiority (NI) randomized clinical trials (RCTs) commonly evaluate efficacy of new antiretroviral (ARV) drugs in HIV patients. Their reporting and interpretation have not been systematically evaluated. We evaluated the reporting of NI RCTs in HIV patients according to the CONSORT statement and assessed the degree of misinterpretation of RCTs when NI was inconclusive or not established.

Methods: PubMed, Web of Science, and Scopus were reviewed until December 2011. Selection and extraction was performed independently by three reviewers.

Results: Of the 42 RCTs (n = 21,919; range 41-3,316) selected, 23 were in ARV-naïve and 19 in ARV-experienced patients. Twenty-seven (64%) RCTs provided information about prior RCTs of the active comparator, and 37 (88%) used 2-sided CIs. Two thirds of trials used a NI margin between 10 and 12%, although only 12 explained the method to determine it. Blinding was used in 9 studies only. The main conclusion was based on both intention-to-treat (ITT) and per protocol (PP) analyses in 5 trials, on PP analysis only in 4 studies, and on ITT only in 31 studies. Eleven of 16 studies with NI inconclusive or not established highlighted NI or equivalence, and distrtracted readers with positive secondary results.

Conclusions: There is poor reporting and interpretation of NI RCTs performed in HIV patients. Maximizing the reporting of the method of NI margin determination, use of blinding and both ITT and PP analyses, and interpreting negative NI according to actual primary findings will improve the understanding of results and their translation into clinical practice.
ABSTRACT FINAL ID: 52

DENGUE VIRUS INFECTION IN HUMAN LUNG: IS CELLULAR TROPISM RESPONSIBLE FOR THE PATHOPHYSIOLOGY OF DENGUE VIRUS INFECTION?

M. Langer, J. Booth, P. Pazoles, E.S. Duggan, J. Mecalf. Internal Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK; A. Srikantiahkocham. Internal Medicine, University of Massachusetts Medical Center, Worcester, MA; K. Coggshall. Immunobiology and Cancer, Oklahoma Medical Research Foundation, Oklahoma City, OK

Abstract Body:

Rationale: Dengue virus (DENV) infection is a major health public threat worldwide. An emerging infectious disease, dengue has been reported in new geographical areas and in those areas where the infection has been previously under control. Infection with DENV causes a wide spectrum of clinical disease ranging from asymptomatic infections to a viral hemorrhagic fever called dengue hemorrhagic fever (DHF), a potentially fatal disease characterized by bleeding tendency and plasma leakage into the abdominal and pleural cavities. The mechanisms underlying vascular leakage in DHF are not well understood, largely due to the lack of animal models that closely mimic human dengue. We hypothesized that pleural mesothelial cells may be more susceptible than alveolar epithelial cells to DENV-mediated effects, leading to disrupted cell-cell integrity.

Methods: To better test the hypothesis, we developed a human lung organ and cell culture model that becomes infected when cultured with DENV. Human lung slices were prepared and primary human alveolar epithelial cells (AEC) were isolated from normal human lungs donated for transplant. Primary human pleural mesothelial cells were isolated from transudative pleural effusions. Cell and human lung slice cultures were exposed to live or UV-inactivated DENV type 2 for 6 hours, washed to remove excess virus, and cultured for various times up to 72 hours before harvesting. Immunofluorescent staining and flow cytometry for DENV antigen was used to determine infection of specific cell types. Quantitative real-time polymerase chain reaction (qRT-PCR) for DENV RNA was used to confirm DENV replication.

Results: Human lung slice cultures supported viral replication as determined by qRT-PCR and infection of specific cell types as determined by immunofluorescent staining. In terms of isolated cell cultures, we found that a higher percentage of mesothelial cells (92%) were infected compared to alveolar epithelial cells (9%) as determined by flow cytometry.

Conclusions: The outcome of these experiments indicates that human pleural mesothelial cells may be more susceptible to DENV infection than alveolar epithelial cells, suggesting selective mesothelial cell disruption by virus infection may be important in the mechanism of several plasma leakage that occurs during DHF. Additionally, our lung organ culture model is useful for further studies of human DENV infection.

ABSTRACT FINAL ID: 64

THE EFFECTS OF TRYPANOSOMA CRUZI INFECTION AND VACCINATION ON PRIMING T CRUZI-SPECIFIC CD4+ T CELLS.

J. Blåse, D. Hoft. Molecular Microbiology and Immunology; Saint Louis University, Saint Louis, MO; C. Eickhoff, D. Hoft. Internal Medicine; Saint Louis University, Saint Louis, MO

Abstract Body:

Background: Trypanosoma cruzi is a protozoan parasite and the causative agent of Chagas disease. The T. cruzi protein trans-sialidase (TS) is currently under investigation as a vaccine candidate. We have generated transgenic (Tg) mice with a monoclonal CD4+ T cell population specific for the TSaa57-74 peptide found to be important in the induction of Tg T. cruzi-specific CD4+ T cells suggest that T. cruzi infection and TS vaccination differentially activate CD4+ T cells. This may explain the defects in CD4+ T cells observed following T. cruzi infection and can perhaps be manipulated.

Methods: To better test the hypothesis, we developed a human lung organ and cell culture model that becomes infected when cultured with DENV. Human lung slices were prepared and primary human alveolar epithelial cells (AEC) were isolated from normal human lungs donated for transplant. Primary human pleural mesothelial cells were isolated from transudative pleural effusions. Cell and human lung slice cultures were exposed to live or UV-inactivated DENV type 2 for 6 hours, washed to remove excess virus, and cultured for various times up to 72 hours before harvesting. Immunofluorescent staining and flow cytometry for DENV antigen was used to determine infection of specific cell types. Quantitative real-time polymerase chain reaction (qRT-PCR) for DENV RNA was used to confirm DENV replication.

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Conclusions: The outcome of these experiments indicates that human pleural mesothelial cells may be more susceptible to DENV infection than alveolar epithelial cells, suggesting selective mesothelial cell disruption by virus infection may be important in the mechanism of several plasma leakage that occurs during DHF. Additionally, our lung organ culture model is useful for further studies of human DENV infection.

ABSTRACT FINAL ID: 72

INFLUENCE OF INDICATION OF CATHETER PLACEMENT ON MICROBIOLOGY OF INDWELLING VENOUS CATHETER INFECTION

L. Shahani, C. Noggle. SIU School of Medicine, Springfield, IL; N. Khardori. Eastern Virginia Medical School, Norfolk, VA

Abstract Body:

Background: Indwelling venous catheters (IVC) have become an integral part of managing patients in both inpatient and outpatient settings. Even with advances in technology and techniques, infectious complications remain highly prevalent and result in significant morbidity and mortality. Various patient and catheter related factors have been previously studied to be associated with IVC related infections. The purpose of this study was to investigate the correlation between the microbiology of IVC related infections and the indication for placement.

Methods: Retrospective review of medical records for hospital admissions due to IVC related complications from October 2008 to December 2010 was conducted. Data regarding the indication for catheter placement and the resulting microbiology was evaluated. Odds ratios and relative risk were calculated with Chi-square analysis determining significance of the association.

Results: Using the Chi-square Continuity Correction, significant associations were found between hemodialysis and the occurrence of Staphylococcus aureus (x2 = 6.359, p = .012), coagulase negative staphylococci (CONS) (x2 = 4.549, p = .033), and other gram positive organisms (x2 = 4.276, p = .039). Antibiotic administration was associated with a significant increased risk of Staphylococcus aureus (x2 = 7.453, p = .006). Total parental nutrition (TPN) administration was associated with an increased risk of Candida spp. (x2 = 8.749, p = .002). In regards to relative risk, individuals presenting with hemodialysis are 1.411 times more likely to present with Staphylococcus aureus, 2.35 times more likely to present with CONS, and 1.397 times more likely to present with another gram positive organism as compared to individuals not on hemodialysis. Individuals undergoing antibiotic administration are 6.2 times more likely to present with CONS and Staphylococcus aureus as compared to individuals not on antibiotics. Finally, individuals with TPN administration are 1.188 times more likely to present with Staphylococcus aureus.

Conclusion: Findings demonstrate that indication of catheter placement can influence variations in the resulting microbiology of IVC infections. These data have implications in choosing initial presumptive antimicrobial therapy and the decision to remove the IVC.

ABSTRACT FINAL ID: 94

OUTCOMES OF ALTERNATIVE ANTIBIOTIC REGIMEN IN PENICILLIN ALLERGIC PREGNANT WOMEN WITH GROUP B STREPTOCOCCAL COLONIZATION OF THE BIRTH CANAL

V. Sundaresan. Infectious diseases, Southern Illinois University, Springfield, IL; P. Vishnumolakala. Department of Family Practice, Southern Illinois University, Springfield, IL; N. Khardori. Infectious Diseases and Microbiology, Eastern Virginia Medical School, Norfolk, VA

Abstract Body:

Background: Group B streptococcal (GBS) colonization of the birth canal is a leading cause of neonatal sepsis. GBS colonization is highly prevalent in asymptomatic pregnant women, with 15-20% of pregant women estimated to have GBS colonization. Because of the high prevalence of GBS colonization, GBS screening in pregnancy is a recommended public health measure. GBS colonization is diagnosed based on obtaining a vaginal discharge culture. The current standard of care for pregnant women with GBS colonization is to administer intrapartum intravenous ampicillin and penicillin G. However, the ideal antimicrobial approach for pregnant women presenting with GBS colonization is not well defined. We previously conducted a cohort study of pregnant women with GBS colonization in which we compared women with and without history of penicillin allergy. In this study, we compared women on an alternative antibiotic regimen (erythromycin) to women on the standard of care antibiotic regimen (ampicillin and penicillin G) to determine if there was evidence of improved maternal or neonatal outcomes.

Methods: This is a single-center, retrospective cohort study of pregnant women with GBS colonization between 2006 and 2010. Women with and without a history of penicillin allergy were identified by chart review. Women with a history of penicillin allergy were randomized to either the alternative antibiotic regimen (erythromycin) or the standard of care antibiotic regimen (ampicillin and penicillin G). Maternal and neonatal outcomes were compared between the two groups.

Results: A total of 186 pregnant women with GBS colonization were identified, of which 92 women had a history of penicillin allergy. Of the women with a history of penicillin allergy, 46 were randomized to the alternative antibiotic regimen (erythromycin) and 46 were randomized to the standard of care antibiotic regimen (ampicillin and penicillin G). There were no significant differences in maternal or neonatal outcomes between the two groups.

Conclusion: This study suggests that an alternative antibiotic regimen (erythromycin) may be a viable option for pregnant women with GBS colonization and a history of penicillin allergy. Further research is needed to confirm these findings and to determine if an alternative antibiotic regimen is more effective in preventing neonatal sepsis.
Abstract Body:

**Background:** Group B Streptococcal (GBS) infections are a leading cause of mortality in neonates acquiring infection through vertical transmission from mothers with vaginal colonization with the bacteria. Newborns may manifest with early-onset infections, late onset infections or infections beyond early infancy as children. It is therefore recommended to screen pregnant women at 35-37 weeks of gestation for colonization (estimated at less than 10%) for administration of prophylactic antibiotics given intrapartum.

One hundred percent of GBS are known to be susceptible to penicillin which is the drug of choice for antibiotic prophylaxis in women colonized with GBS. However in women that are allergic to penicillin, susceptibility testing for clindamycin and erythromycin is performed. Clindamycin or erythromycin is recommended in penicillin allergic women with history of anaphylaxis. Alternatively, vancomycin should be used in penicillin allergic women with clindamycin and erythromycin resistance or unknown susceptibility.

**Objective:** To study the pattern of antibiotics administered as prophylaxis for GBS colonization in pregnant women allergic to penicillin that were admitted to Memorial Medical Center in 2011-2012 as well as compare clinical outcomes in their newborns.

**Methods:** We conducted a retrospective chart review of pregnant women allergic to penicillin and colonized with GBS that were admitted between January 2011 and September 2012 to Memorial Medical Center as well as St. Johns Hospital in Springfield, IL. Outcome measures considered include clinical course in the pregnant women and incidence of early-onset infections in the newborns (bacteremia, sepsisemia without a focus, pneumonia and meningitis). The data on antibiotic susceptibilities was available from Clinical Microbiology Laboratory at Memorial Medical Center.

**Results:** A total of 80 women were identified with stated penicillin allergy. 2013 The American Federation for Medical Research Volume 61, Number 4, April 2013. 777

In cases of unknown allergy to penicillin, there may be a role for penicillin skin testing to rule in true allergy. Despite specific protocols/guidelines and availability of susceptibility data, prophylactic antibiotic choices were not optimal. We also conclude that clindamycin and vancomycin are non inferior to beta-lactams for GBS in prophylaxis since there were no adverse outcomes in the neonates whose mothers received these intrapartum antibiotics.

**Conclusion:** This study elucidates that it is important to obtain a complete and accurate history of penicillin allergy in pregnant women with GBS colonization. In cases of unknown allergy to penicillin, there may be a role for penicillin skin testing to rule in true allergy. Despite specific protocols/guidelines and availability of susceptibility data, prophylactic antibiotic choices were not optimal.

We also conclude that clindamycin and vancomycin are non inferior to beta-lactams for GBS in prophylaxis since there were no adverse outcomes in the neonates whose mothers received these intrapartum antibiotics.

**Table 1**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Number of women that received</th>
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<td>Clindamycin</td>
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<tr>
<td>Vancomycin</td>
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<td>Erythromycin</td>
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**NEPHROLOGY ABSTRACT FINAL ID: 2**

**ACUTE ALDOSTERONE AFFECTS NCC VIA A SPAK-MEDIATED PATHWAY**

B. Ko, L. Hanson. Medicine, University of Chicago, Chicago, IL; A. Mistry, R. Mallick, T. Thai, J. Bailey, J. Klein, R. Hoover. Medicine, Emory University, Atlanta, GA

**Abstract Body:**

The sodium chloride cotransporter (NCC) is a major site of renal sodium reabsorption and plays a key role in blood pressure regulation. Using mDCT15 cells, we previously demonstrated that aldosterone (aldo) increases NCC activity and phosphorylation at 24 hrs. without affecting total or surface NCC abundance. Here we confirm those findings in vivo, examine the mechanisms underlying them and investigate additional time points. Adrenalectomized rats were given aldo or vehicle via osmotic minipumps. Kidney cortex from rats receiving aldo demonstrated no change in total NCC compared to control but showed a three-fold change in phospho-NCC (T53). Similarly, mDCT15 cells showed a three-fold increase in phospho-NCC at 12, 24, and 36 hours corresponding to observed increases in NCC activity as measured by radiotracer uptake with no evidence of changes in total or surface NCC (n=6, p<0.05). mDCT15 cells were treated with shRNA specific for SPAK to generate a cell line with a 68% decrease in SPAK protein expression compared to controls. Stimulation of these cells with aldol had virtually no effect on NCC activity (63%) increase after 24 hour aldol treatment in shRNA SPAK groups versus 52% increase after aldol treatment in control groups, n=4, p<0.01). This provides clear evidence that aldol acutely increases NCC activity without changing NCC abundance or distribution via a pathway involving SPAK and NCC phosphorylation.

**ABSTRACT FINAL ID: 76**

**CLINICALLY RELEVANT DOSES OF ENALAPRIL MITIGATE RADIATION NEPHROPATHY**

E.P. Cohen. Medicine, Medical College Wisconsin, Milwaukee, WI; B.L. Fish, J.E. Moulder. Radiation Oncology, Medical College Wisconsin, Milwaukee, WI

**Abstract Body:**

Mitigation of normal tissue radiation injury is highly relevant for accidental, belligerent, and therapeutic exposures. We have shown mitigation of radiation nephropathy by enalapril 30 mg/liter in the rat drinking water. This dose corresponds to enalapril 16 to 21 mg/m^2/2/d, within the range of usual human doses of enalapril, which is 1.5 to 24 mg/m^2/2/day. Higher dose enalapril is reported to improve outcomes after experimental myocardial infarction and also in subtotally nephrectomized rats. We tested whether enalapril dose escalation improved outcomes in radiation nephropathy. A total body irradiation (TBI) model was used, in forty-eight 7 week old WAG/Rij/CnCr male rats. One leg was not irradiated, to enable marrow autografting. In this model, the major morbidity is renal failure. Enalapril (E) was started five days after TBI, at 30 mg/liter, continued thereafter, or escalated to 60 mg/liter at 12 weeks, and 120 mg/liter at 22 weeks after TBI. There were non-significantly lower systolic BP and BUN at 26 weeks after TBI in rats on escalating doses of enalapril compared to those on fixed dose enalapril, but both were significantly lower at 17 weeks for rats on either enalapril regimen compared to no drug. In our model, rats that have reached a BUN > 120 mg/dl or that are unwell from renal failure must be sacrificed. By this measure of survival, rats on either enalapril regimen had significantly better survival compared to irradiated rats on no drug (p=0.0001), but there was no difference in survival for rats on fixed dose enalapril compared to the escalating enalapril regimen (p=0.7). We conclude that fixed dose enalapril is effective in mitigating radiation nephropathy in a dose range relevant to human use.

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**ABSTRACT FINAL ID: 108**

**MECHANISMS OF OBESITY-ASSOCIATED HYPEROXALURIA: RELEVANCE TO CALCIUM OXALATE KIDNEY STONE DISEASE**


**Abstract Body:**

The majority of kidney stones are composed of calcium oxalate, and minor changes in urine oxalate affect the stone risk. Obesity is a risk factor for kidney stones and obese stone formers often have mild hyperoxaluria. A positive correlation between increased body size and
A 12 month old boy was admitted due to a chief complaint of fever. Physical exam was unremarkable without substantial focal findings. Differential diagnosis was broad and included infectious, neoplastic, and immunological/rheumatological etiologies. Laboratory tests were grossly normal except for a markedly elevated ESR & CRP, monocytosis, and toxic granules in neutrophils. Blood cultures and viral PCR panels were negative. Imaging studies were all normal. His temperature was monitored and controlled with acetaminophen. Fever persisted for 15 days since admission without resolution, until a non-blanching rash with palpable purpura appeared bilaterally on his buttocks and lower extremities. A presumed clinical diagnosis of Henoch-Schönlein purpura was made and the patient was managed symptomatically. A preceding upper respiratory infection could have precipitated this immunological condition. Patient was discharged subsequently without appearance of abdominal, arthralgic or renal symptoms. Patient was to be followed at an outpatient clinic regularly for a high of renal sequel due to the disease.

Conclusion: Fever of unknown origin remains a challenge to clinicians. In this particular patient, a clear cause of fever could not be deciphered until dermatological manifestation of the disease came into sight.

PULMONARY/CRITICAL CARE ABSTRACT FINAL ID: 14
CHARACTERIZATION OF LUNG ENDOTHELIAL CELL ELASTIC PROPERTIES AND GAP CLOSURE IN RESPONSE TO BARRIER-REGULATORY AGONISTS
M.E. Brown, Y. Epsteinstein, S.M. Dudek, J.G. Garcia. Section of Pulmonary, Critical Care, Sleep, and Allergy, Department of Medicine, University of Illinois at Chicago, Chicago, IL; P. Viswanathan, M. Cho. Department of Bioengineering, University of Illinois at Chicago, Chicago, IL.

Abstract Body:
Rationale: Vascular integrity is primarily determined by endothelial cell (EC) cytoskeletal structure that is differentially regulated by endogenous barrier-promoting agents such as sphingosine 1-phosphate (SIP) and hepatocyte growth factor (HGF) and edemagenic agents such as thrombin. Atomic force microscopy (AFM) is a valuable nanotechnology tool employed to characterize structural and mechanical properties in the cytoskeleton of cultured EC in response to barrier regulatory stimuli. In addition, wound healing assays characterize the EC physiological response to physical injury.

Methods/Results: Cultured human pulmonary artery EC (HLMVEC) and human microvascular lung EC (HLMVEC) were used for AFM measurements to determine Young’s modulus under various barrier regulatory conditions based on the Hertz model. SIP induced the highest Young’s modulus increase (6.1KPa) among the barrier enhancing stimuli tested, with HGF(5.8KPa) and FTY720(4.1KPa) inducing lower levels at the 30 minute time point. In contrast, the barrier disruptive agent thrombin decreased from 2.5 KPa to 0.7 KPa depending on the cell type and treatment time. We also developed a wound healing assay enabling quantification of cell migration rates that can be modulated by barrier enhancing agonists (SIP), or by overexpression of the barrier regulatory cytoskeletal protein MLCK (myosin light chain kinase) using adenovirus constructs (±/− stimulation) to compare gap closure rates in HLMVEC. The gap sizes ranged from 37 μm to 357 μm with average migration rates of 150 μm/min for control cells regardless of the presence of SIP, and 1930 μm2/min and 4970 μm2/min for MLCK infected cells with and without SIP stimulation, respectively.

Conclusion: AFM is useful in determining EC biomechanical properties under various barrier regulatory conditions. HLMVEC exhibit higher elastic properties under all conditions compared with HLMVEC. SIP induces higher elastic properties than other barrier enhancing agents tested, while thrombin decreases these properties. Wound assays suggest that MLCK accelerates EC gap closure. This assay is useful for comparing cell migration rates and studying effects of various barrier regulatory agents on gap closure.

ABSTRACT FINAL ID: 18
A SCF E3 LIGASE F-BOX PROTEIN COMPLEX SCFFBXL19 REGULATES RAC1 UBIQUITINATION AND DEGRADATION

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Abstract Body:
Rac1 is a member of the RhoGTPase family, which regulates numerous cellular functions including cell migration, ROS generation, and cytokine release. Rac1 is activated in a GDP bound state and is inactivated in a GTP bound state. The most recent studies have shown that Rac1 stability is regulated by two different E3 ligases dependent on its activation status: active form Rac1 is ubiquitinated by a RING E3 Ligase termed Inhibitors of Apoptosis Proteins (IAPs), while the inactive form of Rac1 is ubiquitinated by HECT E3 Ligase termed HACE1. Here we report that a relatively new F-box protein, termed FBXL19 targets both the active and inactive forms of Rac1 for ubiquitination and degradation in the proteasome system, thus reducing cell migration. Over-expression of FBXL19 directly bound to Rac1 and induced Rac1 degradation. Rac1 stability was increased by FBXL19 silencing. Lysine166 within Rac1 was identified as ubiquitin acceptor site from FBXL19 and lysine166 mutant of Rac1 (Rac1K166R) was resistant to FBXL19-mediated Rac1 ubiquitination and degradation. Further, we found that AKT-mediated phosphorylation of Rac1 is essential for FBXL19-mediated Rac1 degradation. Inhibition of AKT increased Rac1 stability, while over-expression of AKT induced Rac1 ubiquitination and degradation. Unlike the effect on the Rac1 wild type, FBXL19 over-expression had no effect on AKT phosphorylation site mutant of Rac1 (Rac1S71A) ubiquitination and degradation. These findings provide the first evidence of F-box protein targeting Rac1 for ubiquitination and AKT regulating FBXL19-mediated Rac1 depletion.

ABSTRACT FINAL ID: 28

NONMUSCLE MYOSIN LIGHT CHAIN KINASE REGULATES ALLERGIC INFLAMMATION IN A MURINE MODEL OF ASTHMA
T. Wang, L. Moreno-Vinasco, S. Sammani, S.M. Dudek, J.G. Garcia. Department of Medicine, University of IL at Chicago, Chicago, IL

Abstract Body:
Nonmuscle light chain kinase (MYLK) is a multifunctional enzyme involved in isoform-specific non-muscle and smooth muscle contraction, inflammation and vascular permeability, processes directly relevant to asthma. We also demonstrate that the nonmuscle isoform (nmMLCK) expression levels are significantly higher in lung tissue samples collected from asthma patients. Here we further examine the contribution of nmMLCK to asthma susceptibility and pathobiology. We have utilized nmMLCK knockout mice and endothelial cell specific nmMLCK overexpression mice to investigate the role of nmMLCK in murine asthmatic inflammation with an ovalbumin (OVA) model. Mice with targeted nmMLCK deletion were significantly protected from ovalbumin-mediated increases in pulmonary inflammation and airway hyperreactivity, whereas mice with selective endothelial overexpression of nmMLCK exhibited augmented allergic inflammation. Furthermore, as a novel therapeutic strategy, nmMLCK siRNA downregulated lung nmMLCK expression, thereby inhibiting OVA challenge-induced asthmatic inflammation. These data are consistent with the pivotal role of nmMLCK in the regulation of lung endothelial barrier function. The nmMLCK isoform is a novel molecular target in asthmatic pathobiology and asthma susceptibility. This study is supported by NIH grants HL 91899 (JNG), HL 58064 (JKNG), HL 88144 (SMD), and Parker B Francis Foundation (TW).

ABSTRACT FINAL ID: 30

TRANSCRIPTIOINAL REGULATION OF PRE-B CELL COLONY ENHANCING FACTOR GENE BY ACUTE LUNG INJURY ASSOCIATED POLYMORPHISMS
X. Sun, B. Mapes, M. Wade, V. Elangovan, J. Moitra, S. Ma, J.G. Garcia. Medicine, University of Illinois at Chicago, Chicago, IL

Abstract Body:
Rationale: The genetic mechanisms underlying acute lung injury (ALI) susceptibility are poorly understood. We previously reported pre-B-cell colony-enhancing factor (PBEF) to be a pro-inflammatory cytokine, candidate gene and biomarker in sepsis and ventilator induced ALI. Objective: To further explore the transcriptional regulations of PBEF gene and functions of ALI associated polymorphisms. Methods: Genotyping of PBEF polymorphisms was performed using iPLEX Gold reactions. Luciferase activity assays, and chromatin immunoprecipitation assay were utilized to assess the functionality of PBEF promoter and ALI associated polymorphisms.

Results: PBEF promoter variant rs97944560 (-948G/T) is significantly associated with ALI in European Americans from Chicago cohort (71 cases, 186 controls), and rs7789066 (-2422T/C) is significantly associated with ALI in Spanish cohort (66 cases, 96 controls). In silico analysis demonstrated that promoter region of PBEF contains antioxidant response, shear stress and stretch-response elements. 18% cyclic stretch significantly induced transcription factor STAT5 binding to the PBEF promoter, increased its promoter activity and transcription, which was attenuated by silence of STAT5. Promoter activity of PBEF in lung endothelial cells was significantly increased by variant -948G/T and -2422T/C, and further enhanced by 18% cyclic stretch, which were partially or fully blocked by the silence of STAT5.

Conclusion: These data suggest the transcriptional activity of PBEF is regulated by ALI associated genetic variants and STAT5 under mechanical stress, and the interaction of these factors could be a principal mechanism for genetic roles of PBEF in ALI development. (NIHBI HL094394, HL058064).

ABSTRACT FINAL ID: 34

LYSOPHOSPHATIDIC ACID RECEPTOR 2 DEFICIENCY PROTECTS AGAINST BLEOMYCIN-INDUCED LUNG INJURY AND FIBROSIS IN MICE
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Abstract Body:
Rationale: Idiopathic pulmonary fibrosis (IPF) is characterized by alveolar epithelial cell injury, accumulation of fibroblasts/myofibroblasts and deposition of extracellular matrix proteins. Previous study has showed that lysophosphatic acid (LPA) and LPA receptor 1 (LPA1) involved in the lung fibrogenesis; however, the role of other LPA receptors in fibrosis is really unknown.

Methods: Bleomycin (2 U/kg) in PBS was administered intratracheally to LPA2−/− mice or 129/SV control mice. After 0, 3, 7, 14 days, mice were sacrificed, lungs were perfused and fixed for histology and immunohistochemical analysis. Expression of fibronectin (FN), α-smooth muscle actin (α-SMA), and collagen were analyzed by Western blotting. Acid soluble collagen and cytokines were quantified by commercial kits.

Results: LPA receptor 2 (LPA2) deficient mice, compared to wild type controls, were protected against lung injury, fibrosis and mortality in a murine model of bleomycin-induced pulmonary fibrosis. Furthermore, LPA2 deficiency attenuated bleomycin-induced expression of FN, α-SMA and collagen in lung tissue as well as increase of IL-6, total protein and TGF-β1 level in bronchoalveolar lavage fluids. In human lung fibroblast, knock down of LPA2 attenuated LPA-induced expression of TGF-β1 and differentiation of lung fibroblasts to myofibroblasts characterized by decreased expression of FN, α-SMA and collagen via dampened signal transduction of Akt, Smad3 and p38 MAPK. Similarly, knock down of LPA2 with siRNA also mitigated TGF-β1-induced differentiation of lung fibroblast characterized by reduced expression of FN, α-SMA and collagen. Additionally, LPA2 deficiency significantly attenuated bleomycin-induced apoptosis of alveolar and bronchial epithelial cells in mouse lung.

Conclusion: Together, our data suggest a role for LPA2 in bleomycin-induced lung injury and pulmonary fibrosis. This work was supported by NIH P01 HL 98050 to VN.

ABSTRACT FINAL ID: 46

ALTERATIONS IN THE DNA METHYLOMIE OF FIBROBLASTS FROM PATIENTS WITH IDIOPATHIC PULMONARY FIBROSIS
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Abstract Body:
Rationale: Fibroblasts cultured from the lungs of patients with idiopathic pulmonary fibrosis (IPF) have been shown in vitro to exhibit phenotypic features that contribute to excessive matrix production and scarring associated with the disease. The persistence of these features over several passages...
Fibroblasts were cultured from the lung explants of IPF patients. With a threshold of p
IL-6 plays an important role in the inflammatory process of lung tissues from a) control subjects (n=3) and PAH patients. Expression and phosphorylation of Akt/mTOR are increased in P. aeruginosa is an opportunistic pathogen that causes nosocomial pneumonia in critically ill and immunocompromised patients. Our previous studies have revealed up-regulation of NADPH oxidase 2 and 4 in mouse lung tissue and human lung microvascular endothelial cells exposed to

**Results:** With a threshold of p < 0.05, 289 genes were identified to be differentially methylation in IPF fibroblasts compared to nonfibrotic control cells, with a nearly equal number of genes hypermethylated versus hypomethylated in IPF fibroblasts. Gene set enrichment analysis of these genes using ConceptGen identified several concepts overrepresented in annotation in this gene set. The set of differentially methylated genes was independently validated by bisulfite sequencing which showed hypermethylation of the CARD10 and hypomethylation of the MGMT gene promoters in IPF cells. Expression of MGMT and CARD10 transcripts in IPF fibroblasts was variable and inversely correlated with their degree of methylation.

**Conclusion:** IFN fibroblasts are characterized by the aberrant methylation of multiple genes. The altered methylation of certain of these genes, such as CARD10 and MGMT, may contribute to altered expression of genes that contribute to the profibrotic, apoptosis-resistant phenotype of IFP cells.

Funded by: The American Thoracic Society Coalition for Pulmonary Fibrosis and NIH K08 HL094657 01.

**ABSTRACT FINAL ID: 60**

**ACTIVATION OF AKT/MTOR PATHWAY CONTRIBUTES TO ENHANCED PULMONARY ARTERY SMOOTH MUSCLE CELL PROLIFERATION IN PATIENTS AND ANIMALS WITH PULMONARY ARTERIAL HYPERTENSION**

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**Abstract Body:**

**Rationale:** Akt/mTOR signaling pathway is involved in regulating cell growth, proliferation and apoptosis. Our previous studies suggested that inhibition of the Akt/mTOR pathway significantly attenuates store-operated Ca2+ entry (SOCE) and decreases cytosolic free Ca2+ concentration ([Ca2+]_cyt) in pulmonary artery smooth muscle cells (PASMC). An increase in [Ca2+]_cyt in PASMC is a major trigger for pulmonary vasoconstriction and an important stimulus for PASMC proliferation and migration. The aim of this study was to study whether Akt/mTOR pathway is activated in PASMC from patients with pulmonary arterial hypertension (PAH) and animals with experimental pulmonary hypertension (PH), and to examine whether activated Akt/mTOR pathway modulates PASMC proliferation.

**Methods:** Lung tissues from a) control subjects (n=3) and PAH patients (n=3) and b) control mice and mice with hypoxia-mediated PH (HPH), as well as PASMC from 6 healthy controls and 6 PAH patients were used to study changes in Akt/mTOR mRNA and protein levels using Western blotting and real-time PCR, respectively. A BrdU incorporation assay (CellBiosciences) was used to study whether inhibition of mTOR by rapamycin affects human PASMC proliferation during hypoxia (3% O2 for 48 hrs). PDGF (10 ng/ml) was used as a positive control.

**Results:** In human lung tissues, both mTOR protein and mRNA levels were significantly increased in PAH patients compared to controls (protein = 1.45 fold, p < 0.01; mRNA = 2.09 fold, p < 0.05). Phosphorylation of both Akt (p70s6k) by 6.82±2.05 fold, p < 0.01) and mTOR (by 3.25±1.45 fold, p < 0.01) were significantly increased in lung tissues from PAH patients. This study was funded by NIH HL-85064, HL-94394 (J.G.N.G) and 1R25RR032021-01.

**Conclusion:** Expression and phosphorylation of Akt/mTOR are increased in lung tissues and PASMC from patients with PAH and animals with HPH, while inhibition of Akt/mTOR signaling pathway attenuates PASMC proliferation. These data indicate that Akt/mTOR signaling plays an important role in the development and progression of pulmonary vascular remodeling in patients and animals with pulmonary hypertension. Akt/mTOR may be a potential therapeutic target in developing novel treatment for pulmonary hypertension.

**ABSTRACT FINAL ID: 62**

**INTERLEUKIN 6 MODULATES ACUTE LUNG INJURY IN MICE**

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**Abstract Body:**

**Rationale:** IL-6 is a cytokine with both pro- and anti-inflammatory properties. We and others have demonstrated that increased levels of this cytokine are associated with mortality in patients with sepsis and ARDS. Genetic studies suggest that certain IL-6 haplotypes are associated with increased susceptibility to acute lung injury (ALI) in patients. However, because of its complex role in inflammation, the mechanistic effects of IL-6 on ALI are not yet understood.

**Methods:** Utilizing a sepsis-induced model of murine ALI, IL-6 knockout mice and wild type mice (C57Bl/6) were given intratracheal LPS (0.7 mg/kg) and sacrificed 18 hours after dosing. Bronchoalveolar lavage (BAL) was performed at the time of sacrifice, and the BAL fluid was evaluated for total protein, cell count, and white blood cell differential. Lung tissue was fixed in formalin and stained with H&E. For the in vitro studies, human lung microvascular EC and human pulmonary artery EC were incubated with IL-6 with and without its soluble receptor to determine effects on barrier function using measurements of trans endothelial electrical resistance (impedance sensing or ECIS system).

**Results:** BAL protein was ~26% lower in the IL-6 knockout mice than in controls after LPS, indicating that IL-6 expression contributes to the development of lung inflammation and injury in response to LPS. Furthermore, the BAL cell differential in the IL-6 knockout mice demonstrated a 5.3% reduction in number of neutrophils relative to control animals (p < 0.05) despite comparable total cell counts. These results suggest that IL-6 regulates neutrophil migration into the alveolar spaces during the inflammatory response to LPS. ECIS studies demonstrated that IL-6 significantly increased permeability in both human lung microvascular EC and human pulmonary artery EC by ~30% when given in conjunction with its soluble receptor, although no difference was seen when either were given alone. These in vitro results suggest that IL-6 increases lung vascular permeability during inflammation.

**Conclusion:** IL-6 plays an important role in the inflammatory process of ALI by augmenting lung vascular permeability and neutrophil migration into alveoli. These results suggest that modulating IL-6 may be of therapeutic benefit in ALI.

This study was funded by NIH HL-58064, HL-94394 (J.G.N.G) and 1R25RR032021-01.

**ABSTRACT FINAL ID: 70**

**NADPH OXIDASE 4 REGULATES PSEUDOMONAS AERUGINOSA-INDUCED ENDOTHELIAL APOPTOSIS AND PERMEABILITY**

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**Abstract Body:**

**Rationale:** P. aeruginosa is an opportunistic pathogen that causes nosocomial pneumonia in critically ill and immunocompromised patients. Our previous studies have revealed up-regulation of NADPH oxidase 2 and 4 in mouse lung tissue and human lung microvascular endothelial cells exposed to
P. aeruginosa. However, the involvement of Nox protein(s) in Pseudomonas infection is unclear. Here, we investigated the effect of P. aeruginosa infection and Nox proteins on endothelial apoptosis and barrier function in mouse lung and human lung endothelial cells.

Methods: Effect of P. aeruginosa on human lung microvascular endothelial cells (HLMVECs) monolayer permeability was assessed by measurement of trans-endothelial resistance (TER) and Dextran-FITC Transwell Assay. Cell adhesion disruption caused by P. aeruginosa was detected by immunofluorescent staining of VE-Cadherin and Western blot of phosphorylated and total VE-Cadherin. Apoptosis of endothelial cells exposed to P. aeruginosa was monitored by TUNEL staining and detection of Caspase 3 by Western blotting. In vivo, C57BL/6J wild type, Nox2 knockout, or wild type mice treated with siRNA-mediated Nox4 were intratracheally administered 1×10⁶ cfu/mouse of P. aeruginosa strain PA103. At 24 hours after P. aeruginosa infection, pulmonary permeability was assessed by measurement of bronchoalveolar lavage (BAL) fluid protein and total cell count. Lung inflammation was assessed by measurement of BAL inflammatory cytokines, IL-6 and TNF-α.

Results: P. aeruginosa caused a significant increase in TER and Dextran-FITC leakage into the medium, suggesting P. aeruginosa mediated barrier dysfunction. The barrier disruption by P. aeruginosa was inhibited by knockdown of Nox4, but not Nox2. P. aeruginosa induced significant phosphorylation and degradation of VE-Cadherin, which was attenuated by siRNA-mediated knockdown of Nox4, but not Nox2. Analysis of apoptosis by TUNEL staining and cleaved Caspase 3 revealed significant induction of endothelial apoptosis by that P. aeruginosa infection. Also, P. aeruginosa mediated endothelial apoptosis was attenuated by Nox4-siRNA, but not Nox2 siRNA. Compared to wild type mice, airway instillation of P. aeruginosa induced less BAL IL-6 and TNF-α in Nox2 knockout mouse; however, pulmonary leakage as evidenced by BAL protein and cell count was not affected. Nox4 siRNA-treated mouse showed significant decrease in BAL protein and inflammatory cell accumulation, but no amelioration in IL-6 and TNF-α secretion.

Conclusion: These data indicate that Nox2 and Nox4 differentially regulate P. aeruginosa-induced pulmonary permeability and inflammation. Inhibition of Nox4 might be an important therapeutic strategy to attenuate P. aeruginosa induced and endothelial barrier dysfunction.

Abstract Final ID: 78

INHIBITION OF RAC1 GERANYLGERANYLATION PREVENTS THE DEVELOPMENT OF PULMONARY FIBROSIS BY ATTENUATING MITOCHONDRIAL OXIDATIVE STRESS

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Abstract Body:

Rationale: Asbestos is an important cause of pulmonary fibrosis. The generation of reactive oxygen species (ROS), including H₂O₂, by alveolar macrophages plays a critical role in pulmonary fibrosis. We have demonstrated that alveolar macrophages obtained from patients with asbestosis produce high levels of mitochondrial H₂O₂. Asbestos induces import of the GTPase, Rac1, into mitochondria and increases mitochondrial H₂O₂ production. We have also shown that geranylation of Rac1, a post-translational modification, is required for mitochondrial Rac1 import. Based on these observations, we hypothesized that inhibition of geranylation with digeranyl bisphosphonate (DGBP) inhibits mitochondrial H₂O₂ production, and the development of pulmonary fibrosis in vivo.

Methods: Macrophages were exposed to chrysotile asbestos in the presence or absence of DGBP. Whole cells and mitochondrial fractions were isolated and H₂O₂ production measured by H₂O₂ Assay. Wild type (WT) mouse were exposed to chrysotile asbestos, and treated with vehicle (water) or DGBP daily for 21 days. The mice were euthanized, and lung oxidative stress and development of fibrosis were determined. Alveolar macrophages were isolated for measurement of Rac1 expression and H₂O₂ production.

Results: Macrophages exposed to chrysotile asbestos and DGBP in vitro produced less H₂O₂ compared to untreated controls. These results were confirmed for both whole cells and mitochondrial fractions. WT mice exposed to asbestos in the presence of vehicle had increased lung oxidative stress, developed greater lung fibrosis, and had increased collagen deposition compared to WT mice treated with DGBP after asbestos exposure. Alveolar macrophages from vehicle treated WT mice also produced significantly more H₂O₂ and demonstrated greater expression of Rac1 in the mitochondria compared to macrophages from DGBP treated mice.

Conclusion: Rac1 import into the mitochondria of alveolar macrophages is mediated by asbestos exposure and results in increased H₂O₂ production and development of pulmonary fibrosis. Import of Rac1 is modulated by geranylgeranylation, and inhibition of this step with DGBP results in decreased mitochondrial Rac1 expression, decreased H₂O₂ production, and decreased collagen deposition in the lung, which suggests this is a potential therapeutic option for the prevention of asbestos-induced pulmonary fibrosis.

National Institutes of Health grants 2R01ES015981-6, R01ES014871, and a Department of Veterans Affairs Merit Review 1BX801135.

Abstract Final ID: 88

A PROMOTER VARIANT OF SPHINGOSINE 1-PHOSPHATE RECEPTOR 1 ASSOCIATED WITH HUMAN SUSCEPTIBILITY TO SEVERE SEPSIS-INDUCED ACUTE LUNG INJURY

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Abstract Body:

Rationale: The genetic mechanisms underlying acute lung injury (ALI) are poorly understood. We have previously demonstrated that sphingosine 1-phosphate (S1P) and its receptor, S1PR1, are intimately involved in lung inflammatory responses including vascular barrier regulation.

Objective: To explore the functional contribution of S1PR1 gene single-nucleotide polymorphisms (SNPs) to sepsis-induced ALI susceptibility.

Methods: A combination of case-control association study, in silico analysis of gene promoter and promoter functional assay was utilized. We performed association studies in case-control samples of unrelated individuals from both African and European American population in Chicago (218 cases and 378 controls). The effects of SNP on transcription factor binding to S1PR1 were analyzed in silico. S1PR1 promoter was cloned into a luciferase reporter vector and assessed for functionality by luciferase assay in transfected human lung endothelial cells (EC).

Results: In European Americans, a promoter SNP rs79060434 (-616 C/T) significantly influenced the risk of sepsis and sepsis-induced acute lung injury (p=0.05). In silico analysis indicated that -616 C/T significantly influenced the binding of the transcription factor NFkB to S1PR1 promoter. TNF-alpha significantly increased promoter activity of S1PR1 gene in time- and dose-dependent patterns (p=0.05).

Conclusion: These data indicate that S1PR1 promoter variant significantly influences the risk of sepsis and sepsis-associated ALI. (Funded by HL50664 and HL91889).

Abstract Final ID: 92

HYALURONAN-MEDIATED DIFFERENTIAL EXTRACELLULAR VESICLE RELEASE FROM CAVEOLIN-ENRICHED MICRODOMAINS REGULATES SUSTAINED HUMAN PULMONARY VASCULAR INTEGRITY

T. Mirzapoiazova, F.E. Lennon, B. Mambetsariev, P.A. Singleton. Medicine, University of Chicago, Chicago, IL

Abstract Body:

Vascular integrity or the maintenance of blood vessel continuity is a fundamental process maintained through endothelial cell-cell junctions. Defects in endothelial barrier function are an initiating factor in several disease processes including atherosclerosis, ischemia/reperfusion, tumor angiogenesis, cancer metastasis, diabetes, sepsis and acute lung injury. Therefore, understanding the mechanisms of endothelial barrier regulation can provide novel therapeutic strategies. We have previously reported that high molecular weight hyaluronan (HMW-HA), a major glycosaminoglycan in the body, promotes human pulmonary microvascular endothelial cell (HPMVEC) barrier enhancement and is protective in acute lung injury. In contrast, low molecular weight hyaluronan (LMW-HA), produced in disease states by highly activated cells, is toxic to endothelial barrier function. However, the mechanism(s) of the sustained barrier regulation by HA are unknown. Our results indicate that long-term (6-24 hour) exposure of HMW-HA induced release of a specific type of
extracellular vesicle from HLMVEC called enlargeosomes (characterized by AHNAK expression) while LWM-HA long-term exposure promoted release of exosomes (characterized by CD9, CD63 and CD81 expression). These effects were blocked by inhibiting caveolin-microdomain (CEM) formation (MbetaCD). Further, inhibiting exosome release by annexin II shRNA attenuated the sustained barrier enhancing effects of HMW-HA while inhibiting exosomes by dimethyl amiloride (DMA) blocked the sustained barrier disrupting effects of LWM-HA. Finally, exposure of isolated exosomes to HPMVEC monolayers generated barrier enhancement while exosomes led to barrier disruption. Taken together, these results suggest that differential release of extracellular vesicles from CEM modulate the sustained HPMVEC barrier regulation by HMW-HA and LWM-HA.

ABSTRACT FINAL ID: 100

C-ABL INHIBITION DISRUPTS BARRIER FUNCTION IN STRETCHED PULMONARY ENDOTHELIUM AND EXACERBATES MECHANICAL VENTILATION-INDUCED MURINE ACUTE LUNG INJURY

S. Sammani, E. Letsiou, L.M. Vinasco, T. Wang, S. Camp, E. Chiang, S. Dudek, J. Garcia. MED, UIC, Chicago, IL

Abstract Body:
Background: Nonmuscle myosin light chain kinase (nmMLCK) is a critical actin-binding protein and driver of actin cytoskeletal rearrangement that regulates fluid flow in the pulmonary microcirculation, trafficking of inflammatory cells into the lung parenchyma, and alterations in pulmonary vascular permeability. c-Ab1 kinase plays an essential role in vascular barrier regulation via posttranslational modification of nmMLCK. Our previous data demonstrated that Ab1 tyrosine kinase phosphorylates nmMLCK and regulates endothelial barrier function (Dudek et al, Mol Biol Cell, 2010).

Goals and Methods: We hypothesized that Imatinib, which inhibits multiple tyrosine kinases including c-Ab1 and is used as an anticancer agent in multiple malignancies, may exacerbate barrier disruption in cyclic stretched (CS) pulmonary endothelial cells (EC) and lung injury induced by mechanical ventilation (MV) in mice. To explore these hypotheses, human pulmonary artery endothelial cells (HPAECs) were exposed to injurious CS (18% x 24h) and then evaluated for IL-8 secretion and VCAM and VE-cadherin expression by Western blotting. The effects of various concentrations of Imatinib (10, 20, 40 μM) on these parameters were determined.

To explore the effects of c-Ab1 on acute lung injury in vivo, 8-week-old C57BL/6 mice were injected with Imatinib (75 mg/kg) intraperitoneally and subjected to MV-induced acute lung injury (VILIa) (4 h of MV, Vt = 30ml/kg) and monitored for 24 h.

Results: Imatinib significantly increased IL-8 release and VCAM expression in cyclic stretched (18%) HPAECs. In vivo, Imatinib significantly increased bronchoalveolar lavage (BAL) fluid protein levels and total inflammatory cell counts compared to vehicle and sham control groups (*p<0.05 vs. spontaneous breathing mice; **p<0.05 vs. VILI control mice).

Conclusions: These preliminary results indicate that Imatinib exaggerates CS-induced barrier disruption in pulmonary EC and exacerbates MV-induced lung injury in mice, likely via c-Ab1 blockade and possible modulation of nmMLCK activity. These studies have clinical relevance given the extensive use of Imatinib as an anticancer agent and support an important functional role for c-Ab1 in regulating lung vascular permeability in vivo.

ABSTRACT FINAL ID: 104

PATHOLOGIC CYCLIC STRETCH-DERIVED ENDOTHELIAL MICROPARTICLES INDUCE ACUTE LUNG INJURY


Abstract Body:
Introduction: Ventilator-induced lung injury (VILI) is a consequence of mechanical ventilation and contributes to the pathophysiology of lung injury syndromes. The mechanisms that produce or contribute to VILI remain largely unknown. Previously, utilizing an in vitro model of VILI, we demonstrated that human lung endothelial cells (EC) exposed to pathologic cyclic stretch (CS-18%) shed increased numbers of microparticles (MP). As endothelial MP (EMP) have unique composition and function, we sought to characterize EMPs in a murine model of VILI and to explore the effects of CS-18% derived EMPs on development of lung injury in mice.

Methods: C57BL6 male mice were exposed to high tidal volume mechanical ventilation (HV=40ml/kg) for 4 h (in vivo VILI model). Citrated blood was collected by cardiac puncture and processed immediately to obtain platelet free plasma (PFP). PFP was further ultracentrifuged to isolate blood MPs (EMP). To further explore whether MPs are implicated in VILI pathogenesis, mice were injected intratracheally with endothelial MPs (EMP) derived from human pulmonary ECs exposed to CS-18% for 24 h. Lung injury was assessed by bronchoalveolar lavage (BAL) cell counts and protein levels.

Results: The purity and characteristics (size 0.1 to 1 μm) of the isolated EMP population were confirmed by electron microscopy and flow cytometry. In blood derived from HV1-challenged mice, annexin V-positive EMP levels were significantly increased by 82% compared to unventilated controls. Western blotting revealed increased VE-cadherin (endothelial marker) protein levels in MP lysates from VILI-challenged mice compared to controls. In addition, mice receiving intratracheal infusion of CS-18%-EMP displayed significant recruitment of neutrophils and red blood cells into the alveolar space. BAL protein levels were significantly increased (290±10 μg/mL) in EMP-treated mice compared to vehicle-treated mice (127±36 μg/mL).

Conclusion: High levels of circulating EMPs were detected in the blood of VILI mice. Furthermore, our results suggest that EMPs generated during VILI can induce lung injury. Microparticles may represent promising new markers and mediators of lung inflammation.

RHEUMATOLOGY/IMMUNOLOGY/ALLERGY

ABSTRACT FINAL ID: 98

OCULAR AND RENAL INVOLVEMENT AS THE RARE INITIAL MANIFESTATION OF SARCOIDOSIS

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Case Report: A 16-year-old African American male was diagnosed with anterior uveitis after presenting for red painful eyes, and loss of vision in addition to weight loss, fatigue, polydipsia, and polyuria of acute onset. He had no history of hemoptysis, hematochezia, melena, night sweats, fevers, or arthralgias. His past medical and family history was non-contributory. Initial lab work revealed proteinuria, serum Cr of 1.1mg/dl and of ESR-57mm/hr. He was treated with steroid eye drops which improved his vision. Infectious and vasculitic workup was normal with a negative ANA, ANCA, anti-PR3 and anti-MPO antibodies as well negative HIV and TB screening. ACE, 25(OH)D and Ca++ were all normal, a CT chest showed no hilar lymphadenopathy or pulmonary disease. A renal biopsy for rapidly rising serum Cr to 4.7mg/dl over the next three months showed non-caseating granulomatous inflammation with absent acid-fast bacilli and fungal elements by special stains. There was no vascular or glomerular involvement. Treatment with systemic steroids and methotrexate was possible for tubulointerstitial nephritis.

Case Report: A clinical diagnosis of sarcoidosis was confirmed after evaluation for cough and hemoptysis showed new pulmonary micronodular opacities on CT chest and increased ACE levels to 104 (upper limit 101). Its clinical manifestations. In a recent study eye involvement was the initial manifestation of sarcoidosis in 21.2% of patients (2, 3), with anterior uveitis being most common. Renal involvement as an initial manifestation is rare. When present it manifests as proteinuria, hematuria, hypercalciuria, or nephrocalcinosis. Reported patterns of kidney injury are interstitial nephritis with or without granulomatous inflammation on histology or less frequently glomerular disease. Initial presentation with both eye and renal involvement is extremely rare with only a few reported cases.
In summary, this case illustrates the diagnostic challenge of granulomatous oculo-renal inflammation. The differential diagnosis includes TINU, Sarcoidosis, Autoimmune disorders, infections, environmental exposure and neoplasia. A definitive diagnosis may require an extensive work up and close follow up.

References:

Midwestern Regional Meeting Abstracts

Friday, April 26, 2013
Lunch & Closing Poster Session

CARDIOLOGY/CARDIOVASCULAR DISEASE

ABSTRACT FINAL ID: 9

ALTERNATE DAY FASTING WITH A HIGH FAT DIET: IMPACT ON BODY WEIGHT, BODY COMPOSITION, AND CORONARY HEART DISEASE RISK PROFILE IN OBESE ADULTS

M.C. Klempel, C.M. Kroeger, K.A. Varady. Kinesiology and Nutrition, University of IL at Chicago, Chicago, IL.

Abstract Body:
Background: Alternate day fasting (ADF) with a low-fat (LF) diet is effective for weight loss and cardio-protection. However, the applicability of these findings is questionable as most Americans consume a high-fat (HF) diet. This study examined if these beneficial changes in weight and coronary heart disease (CHD) risk can be reproduced if a HF diet is used in place of a LF diet during ADF.

Methods: Thirty-two obese subjects were randomized to an ADF-HF (45% fat) or ADF-LF diet (24% fat), which consisted of two phases: 1) a 2-week baseline weight maintenance period, and 2) an 8-week ADF weight loss period. All food was provided to subjects.

Results: Body weight was reduced (P < 0.001) by ADF-HF (5 ± 1%) and by ADF-LF (4 ± 1%). Fat mass decreased (P < 0.001) by ADF-HF (5 ± 1 kg) and ADF-LF (4 ± 1 kg). Fat free mass remained unchanged. Waist circumference decreased (P < 0.001) by ADF-HF (7 ± 1 cm) and ADF-LF (7 ± 1 cm).

LDL cholesterol and triglyceride concentrations were reduced (P < 0.01) by both interventions (ADF-HF: 18 ± 5%, 14 ± 5%; ADF-LF: 24 ± 3%, 14 ± 4%). The proportion of small LDL particles decreased (P < 0.05) in the ADF-HF and ADF-LF groups by 8 ± 3% and 10 ± 4%.

Conclusion: Thus, an ADF-HF diet produces similar reductions in weight and CHD risk as an ADF-LF diet. These findings are important in terms of diet tolerability and long-term adherence to ADF diets.

ABSTRACT FINAL ID: 11

ALTERNATE DAY FASTING WHEN COMBINED WITH ENDURANCE EXERCISE REDUCES LEPTIN BUT NOT ADIPONECTIN AND RESISTIN

S. Bhatia, M.C. Klempel, C.M. Kroeger, K.A. Varady. Department of Kinesiology and Nutrition, University of IL at Chicago, Chicago, IL.

Abstract Body:
Background: Alternate day fasting (ADF), consisting of a feed day (24-hour ad libitum food intake) alternated with a fast day (75% energy restriction), is effective in reducing body weight and modulating adipose tissue physiology. However, the ability of ADF in combination with endurance exercise to improve the above variables has never been tested.

Objective: Accordingly, this study examined whether the combination of ADF plus exercise produces superior changes in body weight and plasma adipokine levels, when compared to each treatment alone.

Methods: Obese subjects (n=64) were randomized to 1 of 4 groups for 12 weeks: 1) combination (ADF + endurance exercise), 2) ADF, 3) exercise, or 4) control.

Results: Body weight was reduced (P < 0.05) by 7 ± 1 kg, 5 ± 1 kg, and 1 ± 1 kg, and fat mass decreased (P < 0.001) by 6 ± 1 kg, 4 ± 1 kg and 1 ± 1 kg in the combination, A and exercise group, respectively. Fat free mass was retained in all groups. Adiponectin and resistin values did not change in any group post-treatment. Leptin levels significantly decreased (P < 0.05) by 38 ± 10 ng/ml, 15 ± 6 ng/ml and 15 ± 9 ng/ml in the combination, ADF, and exercise group, respectively.

Conclusion: These findings suggest that the combination of ADF plus exercise produces superior changes in body weight, body composition and leptin levels, when compared to each intervention alone.

ABSTRACT FINAL ID: 13

PULMONARY VASCULAR RESISTANCE: A NOVEL METHOD OF NONINVASIVE ESTIMATION AND ASSOCIATION WITH MORTALITY IN CRITICALLY ILL SURGICAL PATIENTS

M. Meltmood, K. Tchorz, R. Markert, M. Chandra, M. McCarthy. Cardiovascular Disease, Wright State University, Dayton, OH.

Abstract Body:
Background: Doppler-derived ratio of peak tricuspid regurgitant velocity (TRV, ms) to the right ventricular outflow tract velocity integral (TVI-ivot, cm) is a reliable non-invasive method of estimating pulmonary vascular resistance (PVR). The easier to measure left ventricular outflow tract velocity integral (TVI-ivot, cm) has been presented as an alternative to TVI-ivot in patients with severe pulmonary hypertension. The correlation of TRV/TVI-ivot with PVR in other patient populations has not been reported.

Methods: This 48-hr study enrolled critically ill and/or injured mechanically ventilated adult surgical patients admitted to a Level I Trauma Centre. Serial pulmonary artery catheter (PAC) and transthoracic echocardiography (TTE) measurements were obtained on each patient every 12 hrs (total – 5 points/patient). Pearson correlation coefficients were obtained between invasive PVR and TRV/TVI-ivot. The Friedman Test determined PVR trends over time. Fisher’s Exact Test determined the association of PVR > 2 Wood’s Unit (WU) with mortality.

Results: Mean age was 49±20 years, 69% were male, and 84% were trauma patients with a mean Injury Severity Score of 24±10. Overall survival rate was 78%. Pulmonary vascular resistance from PAC and TRV/TVI-ivot from TTE were related at each of 5 points in time (correlations 0.44-0.70; P < 0.02). Non-survivors had a progressive increase in PVR over time (2.97, 2.83, 2.87, 3.20, 4.37; p = 0.036) while survivors did not. At 48-hr, non-survivors more frequently had pulmonary vascular resistance > 2 WU (p = 0.023). Using a TRV/TVI-ivot cut-off of 0.16 to predict PVR > 2 WU, the sensitivity and specificity was 75% and 61% respectively during early resuscitation compared to a sensitivity of 50% and specificity of 93% at 48-hr.

Conclusion: Doppler derived TRV/TVI-ivot correlated moderately with PVR in critically ill surgical patients requiring mechanical ventilation. Pulmonary vascular resistance progressively increased amongst non-survivors and PVR > 2 WU at 48-hr was associated with mortality. By utilizing the easier to measure TVI-ivot, TTE may help estimate PVR and identify critically ill patients at risk of death.

ABSTRACT FINAL ID: 15

(PRO)RENIN AND ITS RECEPTOR DURING PREGNANCY AND PREECLAMPSIA: A TRANSLATIONAL APPROACH WITH PATIENTS, A RAT MODEL AND IN VITRO STUDIES

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Abstract Body:
Objective: Preeclampsia (PreE), a syndrome manifesting with hypertension, proteinuria, and edema, is a leading cause of maternal and fetal morbidity and mortality. While preE triggers are likely many and elusive, the renin-angiotensin system (RAS) has been implicated in preE pathogenesis. Most recently it has been demonstrated that the high circulating levels of soluble (pro)renin receptor ([P]RR) at delivery were significantly associated with preE. However, the associations of (pro)renin and ([P]RR) in the pathogenesis of preE remain undetermined.

Study Design: (1) Normal pregnant (n=50) and preE (n=30) patients were recruited from Scott & White Hospital and had their blood drawn between 21 to 40 weeks of pregnancy. Inclusion criteria for determination of preE...
patients include blood pressure >140/90 and presence of proteinuria >300 mg of protein/24 h urine. (Pro)renin levels were assessed in plasma samples by ELISA kit. (2) An established rat model of PreE was used to evaluate the role of (pro)renin and (P)RR in pathogenesis. In this study, we used three groups of animals: nonpregnant control (C, n=10), normal pregnant (NP, n=10); and preE rats (PDS=pregnancy+DOCA+saline, n=10). The preE rats developed hypertension and proteinuria. Blood and urine samples were collected between 18 to 21 days of pregnancy. The plasma and placental levels of RAS components, (pro)renin and (P)RR were assayed in all three groups. The kidney and placental samples from NP and PDS rats were analyzed by immunohistochemistry to evaluate the expression of (P)RR. A tissue signaling MAPK, ERK1/2 phosphorylation was measured in the placenta of NP and PreE rats. (3) Cytotrophoblast (CTB) cells were analyzed for (P)RR expression. The binding of (pro)renin to CTB cells surface (P)RR was evaluated and the interferon-dependent binding by a synthetic decapeptide (resembling the “handle” region of (pro)renin prosegment) were assayed.

Results: (1) The mean plasma (pro)renin of 270.4 ± 100.5 ng/mL in PreE patients differs (p=0.05) from 15 ± 5.5 ng/mL in normal pregnant women. The Receiver Operator Curve analysis of the normal and preE patients data suggests that there is no significant gestational age relationship for (pro)renin in control pregnancies, however, there is a hint from the plot that (pro)renin might increase after 34 weeks of gestation in PreE patients. (2) Active renin, renin and Ang II concentrations in the plasma of PreE rats were lower (p<0.05) than those of NP and C, while placental levels were higher (p=0.05) in PreE rats compared to NP. Both plasma (C: 9 ± 0.3; NP: 12 ± 0.5; PreE: 21 ± 0.3 ng/mL) and placental (pro)renin (NP: 152 ± 4; PreE: 302 ± 69 ng/g tissue) levels were higher (p=0.05) in PreE rats compared to NP. The placental (pro)renin receptor expression and ERK1/2 phosphorylation were higher (p=0.05) in PreE compared to NP rats. (3) Seventy five percentage of (pro)renin was activated by binding to the CTB cells surface (pro)renin receptor and the “decoy” peptide was observed to attenuate this effect.

Conclusions: These data suggest that the peripheral RAS is downregulated and the uteroplacental RAS is upregulated in PreE. However, both circulatory and uteroplacental renin receptor are upregulated in the PreE model. This study provides new evidence that (pro)renin and (P)RR associated novel RAS play key roles in PreE pathogenesis. Moreover, upregulation of ERK1/2 phosphorylation in placenta of rat model indicates the involvement of (P)RR-mediated detrimental cellular signaling in PreE. The attenuation of the binding of (pro)renin to (P)RR on CTB cell by decoy peptide suggests new possibilities to advance drug design for specific blocking agents (for example, (P)RR blockers) with greater benefit in preE.

ABSTRACT FINAL ID: 17
SUPPRESSION OF ALDOSTERONE AND PROGESTERONE IN PREECLAMPSIA: PATIENTS AND A RAT MODEL STUDIES
D. Horvat, D.C. Zwieja. Department of Systems Biology and Translational Medicine, Texas A&M Health Science Center College of Medicine, Temple, TX; S.R. Allen, R.O. Jones, T.J. Kuehl, M.N. Uddin. Department of Obstetrics and Gynecology, Scott & White Healthcare/Texas A&M Health Science Center College of Medicine, Temple, TX; K.G. Pringle. Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, New South Wales, AUSTRALIA; F. Suzuki. Department of Applied Bioscience, Gifu University, Gifu, JAPAN

Abstract Body:
Objective: Preeclampsia (pE) is a hypertensive disorder seen in 3-10% of human pregnancies and is diagnosed by de novo onset of hypertension and proteinuria. Several research groups provided evidence for reduced aldosterone (Aldo) and progesterone (Prog) availability in pE. The aim of this study was to determine the levels of Aldo and Prog in preE.

Methods: Normal pregnant (n=50) and preE (n=30) patients were recruited from Scott & White Hospital and had their blood drawn between 21 to 40 weeks of pregnancy. Inclusion criteria for determination of preE patients include blood pressure >140/90 and presence of proteinuria >300 mg of protein/24h urine. Two groups of rats were used in this study: normal pregnant (n=10) and preE rats (n=10) which were given weekly injections of desoxycorticosterone acetate and 0.9% saline to drink. The preE rats developed hypertension and proteinuria. Aldo and Prog levels were assayed in plasma and urine samples by commercially available ELISA kits. Results: In preE patients, the mean Aldo (398.6 ± 115.8 pg/mL) and Prog (46.2 ± 9.5 ng/mL) levels were significantly suppressed (p=0.05) compared to normal pregnancies (Aldo: 548.4 ± 173.5 pg/mL, Prog: 82.5 ± 13.6 ng/mL). Normal pregnancies exhibited a trend of increased levels of Aldo and Prog with an increase in gestational age, however preE patients had opposite trend. Both plasma and urinary levels of Aldo (NP: 578.5 ± 152.4, PDS: 379.5 ± 89.6, NPM: 348.7 ± 91.3 pg/mL for plasma; NP: 86.7 ± 10.7, PDS: 52.0 ± 5.2, NPM: 58.9 ± 6 for urine, p<0.05 for each case) & Prog (NP: 31.5 ± 2.9, PDS: 18.6 ± 2.1, NPM: 16.5 ± 91.3 ng/mL for plasma; NP: 6.5 ± 0.5, PDS: 3.5 ± 0.4, NPM: 3.2 ± 0.5 for urine, p<0.05 for each case) were significantly lower in PDS and NPM rats compared to NP.

Conclusions: We have demonstrated that both Aldo and Prog were suppressed in urine and plasma in a rat model of preE as well as plasma in patients with preE, which may be used as biomarkers for the severity of preE.

ABSTRACT FINAL ID: 33
ANGIOGENESIS IN MOUSE ISCHEMIC HINDLIMB: ROLE OF SEROTONIN TYPE 4 RECEPTOR
J. Profirovic, St. Louis College of Pharmacy, St. Louis, MO; J. Profirovic, E. Stedalova, N. Urao, A. Kehanovic, M. Uskio-Fukai, T. Voyno-Yasenetskaya. University of Illinois, Chicago, IL

Abstract Body:
Serotonin (5-hydroxytryptamine, 5-HT) type 4 receptor (5-HT4R) regulates many physiological processes, including learning and memory, cognition, and gastrointestinal motility. Little is known about its role in angiogenesis. We examined the role of 5-HT4R using in vivo and in vitro experimental models. Using mouse hindlimb ischemia model of angiogenesis, we observed a significant reduction of limb blood flow recovery 14 days after ischemia and a decrease in density of CD31-positive vessels in adductor muscles in 5-HT4R-/- mice compared to wild type littermates. Our in vitro data indicated that 5-HT4R endogenously expressed in endothelial cells (ECs) may promote angiogenesis. Inhibition of the receptor with 5-HT4R antagonist RS 59604 reduced EC capillary tube formation in the reconstituted basement membrane. Using Boyden chamber migration assay and wound healing “scratch” assay, we demonstrated that RS 59604 treatment significantly suppressed EC migration. Transendothelial resistance measurement and immunofluorescence analysis showed that a 5-HT4R agonist RS 67333 led to an increase in endothelial permeability, actin stress fiber and interendothelial gap formation. Importantly, we provided the evidence that 5-HT4R-regulated EC migration may be mediated by Gα13 and RhoA. Our results suggest a prominent role of 5-HT4R in promoting angiogenesis and identify 5-HT4R as a potential therapeutic target for modulating angiogenesis under pathological conditions.

ABSTRACT FINAL ID: 35
MITRAL VALVE PAPILLARY FIBROELASTOMA: A RARE CAUSE OF ACUTE MYOCARDIAL INFARCTION
M. Mehmood, G.T. Broderick. Cardiovascular Disease, Wright State University, Dayton, OH; G.T. Broderick, M.K. Saleh. Good Samaritan Hospital, Dayton, OH

Case Report Body:
Introduction: Primary tumors of the heart are rare. Fibroelastoma, the commonest valvular tumor, presents an embolic risk. ST-segment elevation myocardial infarction (STEMI) due to embolization of fibroelastoma can be a diagnostic challenge and has been infrequently described. We discuss a case of mitral valve papillary fibroelastoma presenting as STEMI.

Case Presentation: A 38-year-old African American female with history of peptic ulcer disease and recreational marijuana use presented with acute onset anginal chest discomfort. Troponin I was mildly elevated and ECG showed subtle evolving ST-segment elevations in leads V2-V4. Urgent coronary angiography revealed total abrupt “cutoff” occlusion of the distal left anterior descending artery (LAD) and a small 3rd diagonal branch suggestive of distal embolization. Balloon angioplasty of the distal LAD resulted in incomplete restoration of flow. Bolus intracoronary Eptifibatide yielded no further angiographic improvement. A transesophageal echocardiogram demonstrated a small mass in the posterior mitral valve leaflet. Differentials of torn chordae tendineae and possible endocarditis were entertained in the report. Transesophageal echocardiography confirmed a 0.66 x 0.59 cm rounded pedunculated structure attached to the posterior mitral leaflet. The patient underwent minimal invasive tumor excision with preservation of the mitral

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valve. Histology confirmed papillary fibroelastoma. Recovery was uneventful and the patient was doing well at 2-month follow-up.

Discussion: Papillary fibroelastoma, a benign cardiac tumor, can be symptomatic due to mechanical effects of the tumor or distal embolization. Surgical excision is recommended due to high risk of embolization.

Conclusion: Coronary artery embolization of fibroelastoma is a rare but well-recognized risk.

ABSTRACT FINAL ID: 41
THE CRUCIAL ROLE OF PECAM1 INDUCED BY SIMVASTATIN FOR ENDOTHELIAL BARRIER FUNCTION
W. Chen, M. Habeck, X. Ni, J.G. Garcia, J.R. Jacobson. Medicine, University of IL at Chicago, Chicago, IL
Abstract Body:
Rational: Platelet endothelial cell adhesion molecule (PECAM) plays an important role neurophil adhesion and responding to inflammatory stimuli, endothelial cellular junction integrity. Statin is HMG-Coenzyme reductase inhibitor and has an outstanding anti-inflammatory effect and endothelial barrier functional enhancement effect. Previous studies indicated that atorvastatin-mediated PECAM-1 expression increase contributed to endothelial barrier functional enhancement effect, but the mechanism has not well characterized.
Methods/Results: In this study, our results confirmed that simvastatin had similar PECAM-1 induction effect in human pulmonary artery endothelial cells (HPAECs) at mRNA and protein level. At same time, simvastatin-mediated PECAM-1 induction enhanced endothelial barrier function by ECIS and monolayer HPAECs permeability measurement. Silence of PECAM-1 diminished endothelial barrier protective effect of simvastatin. Promoter activity assay showed that simvastatin-directed target sequence located in promoter 788-688 nucleotides region. Transcriptional factor binding sequence assay showed that GATA(R) and C/EBPβ potentially bind these target sequence of PECAM-1 promoter. Deletion of GATA(T) and C/EBPβ target sequence from PECAM-1 promoter showed that simvastatin-induced PECAM-1 promoter activity significantly decreased at condition of deletion of C/EBPβ binding sequence but not for GATA(R) binding sequence. Microarray and western blotting study showed that simvastatin induced increase of C/EBPβ as time dependent manner and significantly increase at 24 hours and reach to maximum at 48 hours. Cytosolic and nuclear fraction protein assay showed that 16 hours simvastatin treatment of HPAECs significantly increase C/EBPβ in both of cytosolic and nucleus HPAECs. Moreover, silence of C/EBPβ diminished simvastatin-mediated induction effect of PECAM-1 in HPAECs.
Conclusion: Simvastatin-mediated PECAM-1 induction plays an important role in endothelial barrier enhancement effect. Simvastatin induced PECAM-1 expression in HPAECs through induction of C/EBPβ signal pathway.

ABSTRACT FINAL ID: 43
REGULATION OF CONNEXIN43 AND ARRHYTHMOGENESIS BY MICRORNA-130A
A. Osborne, S. McSharry, H. Padden, M. Broman, J. Earley, G. Kim. Department of Medicine, University of Chicago, Chicago, IL
Abstract Body:
Purpose: MicroRNAs (miRs) are endogenous ~22 nucleotide RNA molecules that are now recognized as critical regulators of diverse physiological and pathological processes including proliferation, apoptosis, differentiation, metabolism and development. These regulatory effects of microRNAs are affected by their repression of specific messenger RNAs. Up to one-third of human genes are predicted to be regulated by one or more microRNAs. Despite this vast regulatory network and hundreds of microRNAs known to date, relatively few targets have been validated, particularly in cardiac disease. MicroRNA-130a is expressed in a tissue-restricted pattern and dynamically regulated in the developing heart. We have shown that transgenic mice that over-express microRNA-130a during cardiogenesis using the β-myosin heavy chain (MHC) promoter demonstrate embryonic lethality due to a variety of cardiac defects.
Methods: Utilizing a cardiac-restricted inducible overexpression transgenic mouse model, we induced microRNA-130a overexpression in the mouse heart under the control of an inducible α-MHC promoter.

Results: With this model, induction of microRNA-130a over-expression in the adult heart resulted in the development of a progresive decline in ventricular function. Fractional shortening was reduced (20% ± 5.3 vs 38% ± 3.2, p<0.001) at 16 weeks post birth. Prior to the decline in cardiac function, we noted a loss of sinus rhythm as detected on screening ECGs performed at 12 weeks post birth. Surface ECG was suggestive of atrial fibrillation and no atrial contractions were evident by pulse wave Doppler. These findings were confirmed by intracardiac recordings. Interim nonsustained ventricular tachycardia was also observed on surface ECG recording. A computational analysis of target messenger RNAs using the target prediction algorithms, PicTar, Microcosm, and TargetScan, revealed several genes with known functions in cardiac conduction and arrhythmogenesis including connexin43. Western blot analysis of adult hearts confirmed significantly reduced protein level of connexin43. Luciferase assays in T73 Fibroblasts, known to express miR-130a, demonstrated significantly reduced luciferase activity in those constructs with the connexin43 3`UTR. Immunofluorescence staining revealed a near complete loss of connexin43 in both atrial and ventricular tissues compared to non-transgenic littermate control hearts. Electron microscopy confirmed a loss of gap junctions in the cardiomyocytes visualized.

Conclusions: Taken together, miR-130a is an important regulator of normal cardiac conduction via regulation of genes such as connexin43. Dysregulation of miR-130a results in the development of a variety of cardiac arrhythmias.

ABSTRACT FINAL ID: 57
SEARCHING FOR BIOMARKERS IN HEART FAILURE WITH PRESERVED EJECTION FRACTION
S. Mirza, M. Chesnik, J. Meskin, C. Gastonguay, J.L. Strande. Medical College of Wisconsin, Milwaukee, WI
Abstract Body:
Background: Half of patients with heart failure (HF) have a preserved left ventricular ejection fraction (HFpEF). Morbidity and mortality are similar to values observed in patients with HF due to reduced EF, yet no effective treatment has been identified for HFpEF. Often the diagnosis of HFpEF is challenging and relies upon careful clinical evaluation, echocardiography with Doppler, and invasive hemodynamic assessment. There is a pressing medical need for minimally invasive objective metrics in order to assist in the diagnosis, monitor therapy and intervene before irreversible cardiac damage is present. The overall aim of this study is to identify a proteomic-based peripheral blood biomarker panels in a serial and prospective monitoring strategy in HFpEF patients. We hypothesize that pathogenesis of HFpEF involve mechanisms that will be reflected in the platelet proteome. For this study, we used a mass spectrometry based approach to perform the initial discovery stage of biomarker development for the identification of HFpEF.

Methods: To qualify for the study, subjects needed to have New York Heart Association Class II-IV heart failure symptoms that required hospitalization, a left ventricular EF >50%, and evidence of diastolic dysfunction. An E/E' ratio > 15 provides stand-alone evidence of diastolic dysfunction, whereas an elevated dp/dt needed to be associated with an E/E' > 8 m/s a mithral flow velocity Doppler signal showing an E/A ratio <0.5. 50% deceleration time > 280 ms, a left atrial size >40 mL/m2, or an left ventricular mass >149 g/m2 or 122 g/m2 (women). Subjects were excluded if they had a clinical condition that would potentially change plasma biomarker profiles independent of the presence of HFpEF. Inactivated platelets were isolated from blood samples. Platelet proteins were extracted, fractionated, and subject to tandem mass spectrometry. Proteins that showed significant changes in expression between symptomatic and asymptomatic subject, but were not observed in control samples were analyzed with Ingeny Systems IPA software.

Results: Analysis of three samples (1) Control subject, (2) Symptomatic HFpEF subject and (3) Asymptomatic HFpEF subject identified 1542 unique proteins with ≥5 scans with peptide probabilities of ≥0.85. Of these, 158 proteins were unique to the HFpEF subject when symptomatic vs. when asymptomatic and vs. the control subject. In particular, our data shows that MYH6, representing alpha-myosin heavy chain; a sarcomeric protein only expressed in cardiomyocytes, was found in the platelet proteome of a subject with HFpEF and up-regulated during a symptomatic HF episode.

Conclusion: This indicates that the pathogenesis of HFpEF can be reflected in the platelet proteome. The identification, development and validation of
ENDOCRINOLOGY/METABOLISM

ABSTRACT FINAL ID: 31

13C-GLOCOSE BREATH TESTING PROVIDES A NON-INVASIVE MEASURE OF INSULIN RESISTANCE


Abstract Body:

Objective: To evaluate the correlation of a simple noninvasive breath test based on a standard oral glucose tolerance load with whole-body glucose disposal rate measured by gold standard hyperinsulinemic euglycemic clamp.

Research Design and Methods: A total of 68 men and women, age 18-65, categorized as non-diabetic lean and obese or obese type 2 diabetic subjects, studied on 2 consecutive days under fasting conditions. On the first day, a 75 g oral glucose load spiked with a small amount of 13C-glucose was administered with subsequent breath sampling over 3 hours. The next day, a hyperinsulinemic euglycemic clamp was performed. Correlations between breath parameters and clamp-derived glucose disposal rate were evaluated using Pearson correlations, and these correlations were compared using the Fishers r-to-Z transformation.

Results: The characteristics of the study participants are described in Table 1. The groups differed in all breath test parameters, with a graded reduction in tracer enrichment with increasing obesity/diabetes. Breath test parameters were strongly correlated with clamp-derived glucose disposal rate with the largest r values observed between clamp-derived glucose disposal rate (GDR) and the single measurement of breath enrichment taken at 180 minutes post ingestion (r=0.612, p<0.0001). Significant correlations of the breath enrichment with GDR were also observed at 90 minutes post ingestion (r=0.543, p<0.0001) and at 120 minutes post ingestion (r=0.580, p<0.0001). Pairwise comparisons of the breath test correlations with GDR using Fishers r-to-Z transformation showed that all correlations were statistically similar. The correlations of the GDR with breath-derived measures were comparable in magnitude and not statistically different from the correlation of GDR with HOMA-IR (r=0.678, p<0.0001).

Conclusions: 13C2 oxidation in exhaled breath following a standard oral glucose load with added 13C-glucose is a strong correlate of whole-body insulin resistance. This correlation was observed over a broad range of glucose tolerance relevant to studies of diabetes. This noninvasive breath-test based approach may provide a useful surrogate measure of whole-body insulin resistance in physiologic and epidemiologic studies. Future studies are needed to develop clinical applications taking advantage of the noninvasive nature of this testing approach.

Table 1. Population characteristics

<table>
<thead>
<tr>
<th>Age group</th>
<th>GDR (mg/min)</th>
<th>HOMA-IR</th>
<th>Breath CO2 (%)</th>
<th>Breath CO2 (mg/min)</th>
<th>Breath CO2 (mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young adult (22)</td>
<td>39.2 12.6 46.2 9.3 49.0</td>
<td>7.3</td>
<td>0.68 (0.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle aged (43)</td>
<td>51.9 15.5 55.8 13.6 50.5</td>
<td>8.7</td>
<td>0.068 (0.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Older aged (64)</td>
<td>70.4 15.7 75.4 13.7 64.5</td>
<td>8.7</td>
<td>0.068 (0.03)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: All correlations were statistically similar. The correlations of the GDR with breath-derived measures were comparable in magnitude and not statistically different from the correlation of GDR with HOMA-IR (r=0.678, p<0.0001).
ABSTRACT FINAL ID: 51
UNEXPLAINED HYPOTENSION IN THE INTENSIVE CARE UNIT: EUTHYROID SICK OR SICK HYPOTHYROID – A LESSON FROM TWO CASES
K. Ghaireeb, C.A. Koch, R. Avusula, T. Fülpö, University of Mississippi Medical Center, Jackson, MS; É. Csongrádi. University of Debrecen, Debrecen, HUNGARY
Abstract Body:
Unexplained hypotension in the intensive care unit (ICU) setting is commonly attributed to volume depletion, cardiac failure, sepsis and/or relative adrenal insufficiency. Hypothyroidism usually associated with hypotension in non-ICU patients is considered less frequently in the differential diagnosis. As interpretation of thyroid testing results is more challenging in the critically ill patients, diagnosis may be delayed, resulting in potential harm to these patients.

First Case: a 71 year-old African American (AA) female with stage 4 CKD, hypertension (HTN) and type-2 diabetes mellitus (DM-2) was admitted for uncontrolled hyperkalemia and hypothermia. She responded initially well to IV fluids, antibiotics and empirical steroids but experienced persistently suppressed mental status, relative hypothyroidism and difficulties with weaning from mechanical ventilation. Thyroid function testing returned with only modest elevation of Thyroid Stimulating Hormone (TSH) of 7.65 µIU/mL [0.27-4.20], but free T4 was low at 0.46 ng/dl [0.93-1.70]. After a trial of IV levothyroxine (100 mcg/day), blood pressure (BP) rapidly rose, mental status improved and she was weaned from the respirator with ease. Further clinical follow-up ruled out central hypothyroidism.

Second Case: a 60 year-old AA male with DM-2, obstructive sleep apnea (OSA), and HTN who arrived with septic shock, respiratory failure and renal failure secondary to S. hemolyticus bacteremia. He maintained a fair urine output despite being dependent on norepinephrine to maintain BP. TSH returned in normal range: 2.52 µIU/mL but free T4 was low: 0.46 ng/dl. After initiation of IV levothyroxine (75 mcg/day), blood pressure (BP) rapidly rose, mental status improved and she was weaned from the respirator with ease. Further clinical follow-up ruled out central hypothyroidism.

Discussion and Conclusion: TSH is commonly suppressed in otherwise euthyroid but critically ill patients. Therefore, seemingly “normal” or only mildly abnormal TSH values in a critically ill ICU patient are worrisome for a potential hypothyroid state. The usual threshold for abnormal laboratory values in diagnosing myxedematous coma may not apply under such circumstances; the concept of “thyroid insufficient state” may better describe these circumstances. The presence of co-morbid OSA may represent a further barrier hindering successful weaning from mechanical ventilation. Early recognition and treatment may assist with recovery from hypotension or from a mechanically-ventilated state. Rather than attributing all abnormal thyroid tests to “euthyroid sick” syndrome, these patients may need careful individual assessment, incorporating all the co-existing medical concerns in the potential pathogenesis of hypothyroidism-induced or further aggravated existing cardiomyopathy. The concept of relative thyroid insufficiency in the ICU setting needs additional studies and exemplifies individualized medicine.

ABSTRACT FINAL ID: 53
INSULIN SIGNALING MODULATES PROINSULIN PROCESSING BY REGULATING TRANSLATION INITIATION IN PANCREATIC β-CELLS
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Abstract Body:
Hyperproinsulinemia is among the early features observed in the pathogenesis of Type 2 Diabetes (T2D). The β-cell is unique in its ability to process immature proinsulin into bioactive insulin using prohormone convertases PC1/3, PC2 and Carboxypeptidase E (CPE). Insufficient action of either of these enzymes leads to accumulation of unprocessed proinsulin. To explore the role of insulin action in proinsulin processing we examined mice lacking functional insulin receptors in β-cells (βIRKO). βIRKO mice, primary islets and β-cell lines exhibited increased circulating and cellular proinsulin content. Concomitantly, we observed that βIRKO β-cells exhibit dilated endoplasmic reticulum (ER) and concordant increase in expression of unfolded protein response including phospho- and total IRE1α, BiP spliced and total XBP1, suggesting compensation for the increased proinsulin biosynthesis. Increased proinsulin content was associated with reduced (~60%) expression of CPE due to a decrease in CPE protein biosynthesis but not alterations in transcripts or protein stability. Polyribosome profile analysis from β cell lines treated with thapsigargin to mimic ER stress revealed a shift in CPE mRNA from polyribosomes to monoribosomes in βIRKO, indicating a block in CPE translational initiation. Interestingly, we observed downregulation of the translational initiation scaffolding protein eIF4G1 in βIRKO cells, which was confirmed by acute knockdown of the insulin receptor in control β-cells suggesting direct regulation by insulin signaling. Further, we showed for the first time that eIF4G1 expression is coordinately regulated by Pax1 and SREBP1 transcription factors. Re-expression of the insulin receptor, knocking down proinsulin, restoring the levels of CPE or eIF4G1 each independently reversed the phenotype. Thus, we provide evidence for the first time to our knowledge, direct regulation of key proinsulin processing enzyme, CPE, by insulin signaling via inhibition of translational initiation that is mediated by Pax1 and SREBP1. Together these data provide compelling evidence for a novel link between insulin signaling, translational initiation, insulin processing and ER stress in β-cells.

ABSTRACT FINAL ID: 55
OCULAR THROMBOSIS, A WINDOW TO FAMILIAL THROMBOPHILIA
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Abstract Body:
Objective: To assess contributions of familial thrombophilia to ocular thrombosis, prospective studies of thrombophilia were done in 90 consecutively referred patients with ocular thrombosis, 69 with retinal vein thrombosis (RVT), 14 with retinal artery thrombosis (RAO), and 7 with amaurosis fugax (AF).

Design: Thrombophilia was compared in patients and in 105 healthy controls without ocular thrombosis. PCR measures were made for Factor V Leiden, Prothrombin, MTHFR C677T-A1298C, and plasminogen activator inhibitor 4G4M mutations. Serologic tests were done for antigenic anti-thrombin III, proteins C and S (total and free), homocysteine, Factors VIII and XI, the lupus anticoagulant, anticardiolipin antibodies IgG and IgM, and protein C and S. Plasminogen activator inhibitor activity was assessed.

Setting: Outpatient clinical research center

Patients: 90 (55 women, 35 men, 84 white, 5 black, 1 other), mean ± SD age 53 ± 17.3 years, 69 RVO (x women, y men), 14 RAO (x women, y men), 7 AF (x women, y men).

Results: Serum homocysteine was high in 36% of RTA (p=.0002) and in 19% of RVT (p=.0001) compared to healthy controls (2%). Factor VIII was high in 29% of RVT vs 7% of controls, (p=.031). Lp (a) was high in 29% of AF vs 20% of controls (p=.022).

MTHFR C677T homozygosity or compound C677T-A1298C heterozygosity was present in 8% of controls vs 29% of RTA patients (p=.008) and 14% of RTA patients (p=.022)

Conclusions: Familial thrombophilia is common in RVT (high homocysteine, high Factor VIII) and RTA (high homocysteine). Homocysteine can be normalized by treatment with folic acid-vitamin B6-vitamin B12. Thrombophilia testing in ocular thrombosis is indicated. Undertreatment of homocysteine, Factor VIII, or Factor VIII in patients at high risk for recurrent ocular thrombosis, and at high risk for other venous and arterial thrombi, and opens avenues to family screening.

ABSTRACT FINAL ID: 59
FOXP3 MUTATIONS CAUSING NEONATAL DIABETES WITHOUT CLASSIC FEATURES OF IPEX SYNDROME
I.L. Hwang, H. Ye, V.P. Paz, A. Pastore, L.H. Philipson, S.W. Greely. Section of Adult and Pediatric Endocrinology, Diabetes, and Metabolism, The University of Chicago, Chicago, IL; X. Liu, C.L. Hanis. Genetics Center, University of Texas Health Science at Houston, Houston, TX
Abstract Body:
Hyperproinsulinemia is among the early observed in the pathogenesis of Type 2 Diabetes (T2D). The β-cell is unique in its ability to process immature proinsulin into bioactive insulin using prohormone convertases PC1/3, PC2 and Carboxypeptidase E (CPE). Insufficient action of either of these enzymes leads to accumulation of unprocessed proinsulin. To explore
Abstract Body:
Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a rare autoimmune disorder caused by mutations in FOXP3, a gene encoding a forkhead box transcription factor required for the function of CD4+ CD25+ regulatory T cells which regulate immunologic tolerance. In addition to neonatal diabetes, classic IPEX includes severe autoimmune enteropathy and eczema. Even with aggressive immunosuppressive treatment or stem cell transplant, death often occurs by 24 months of age from failure to thrive or sepsis. We describe the variable phenotypes of subjects unexpectedly found to have FOXP3 mutations within our Neonatal Diabetes Registry (https://monogenicdiabetes.uic.edu/neonatal-registry/).

Exome sequencing of 19 probands without a known cause for neonatal diabetes revealed 3 different FOXP3 mutations in 4 male subjects in 3 families. One mutation is novel (R114W). Clinical information is shown in Table 1 (007NC01 and 007NC04 are brothers).

We conclude that mutations in FOXP3 are not uncommon in patients with neonatal diabetes even when they lack other features of IPEX syndrome. Our prevalence is comparable to a previous report of FOXP3 mutations found in 6.7% of confirmed monogenic cases. The four cases we describe have only mild inconsistent IPEX-like features, are currently 7 to 31 years old and have never been treated with immunosuppressive treatments. Mutations in FOXP3 should be considered in male patients with early onset diabetes with or without other features.


Acknowledgments: This work has been funded by grants from the American Diabetes Association: 1-11-1CT-41; the University of Chicago Diabetes Research and Training Center: P60 DK020595; the Juvenile Diabetes Research Foundation: 9-2008-177, as well as the U.S. National Institutes of Health Clinical and Translational Science: UL1RR024999.

Table 1 Clinical Characteristics of Subjects with FOXP3 Mutations

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<tr>
<th>FOXP3 mutations</th>
<th>007NC01</th>
<th>007NC02</th>
<th>007NC03</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Previous report</td>
<td>Multiple reports of various phenotypes</td>
<td>None</td>
<td>One report with classic IPEX</td>
<td>One report</td>
</tr>
<tr>
<td>Current age (y)</td>
<td>18</td>
<td>31</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Gestational age (w/d)</td>
<td>42</td>
<td>40</td>
<td>45</td>
<td>37.5</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3900</td>
<td>3950</td>
<td>3520</td>
<td>3400</td>
</tr>
<tr>
<td>Diabetes age (y)</td>
<td>3.5</td>
<td>26.1</td>
<td>194.3 (2.1 y)</td>
<td>28.7</td>
</tr>
<tr>
<td>Diabetes treatment</td>
<td>Insulin pump</td>
<td>Insulin pump</td>
<td>Insulin pump</td>
<td>Insulin pump</td>
</tr>
<tr>
<td>LAP-308 allele (%)</td>
<td>9.3</td>
<td>7.8</td>
<td>7.9</td>
<td>8.4</td>
</tr>
<tr>
<td>Autoimmune, antibody status</td>
<td>No antibody available</td>
<td>anti-thyroid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>No symptoms</td>
<td>Mild chronic cholecystitis</td>
<td>Extreme IFF, weight &lt; 50% percentile, EGI showed inactive pancreatic ducts and cholestasis</td>
<td>No symptoms</td>
</tr>
<tr>
<td>Skin</td>
<td>Mild eczema</td>
<td>Mild eczema</td>
<td>Severe eczema, vitiligo</td>
<td>Mild eczema</td>
</tr>
<tr>
<td>Other medical problems</td>
<td>Short stature, frequent upper respiratory infections associated with wheezing</td>
<td>Hypothyroidism</td>
<td>Friedreich ataxia with degeneration of sensory rhomboid cells, ambiguous hypoplasia and pupillary reflex, vision problems, one eye hypoplasia</td>
<td>Vitamins B, D, E deficiency</td>
</tr>
<tr>
<td>Immune suppression</td>
<td>Never</td>
<td>Never</td>
<td>Never</td>
<td>Never</td>
</tr>
</tbody>
</table>

HOMA insulin resistance (IR), plasmogen activator inhibitor activity (PAI-Fx), HDL and LDL cholesterol (HDLC, LDLC). Women with high levels of both ALT (55 U/L) and AST (>40 U/L) were compared to women with normal ALT, and bilirubin (≤ 1.2). In the absence of other hepatic insults high ALT and high AST are markers for non-alcoholic fatty liver disease (NAFLD). ALT, AST, and other measured variables were determined pre-treatment and after 12.6 months on metformin 2.5 g/day and a low glycermic index (GI) diet.

Results: Of 1195 women (0.34%) had both ALT and AST high, and 999 (92.9%) had normal AST, ALT, and bilirubin. The 40 with high AST and ALT had higher BMI 37.5 ± 5.7 vs 35.1 ± 9 vs normals, and after covariance adjusting for race, age, and BMI, had higher insulin 34.4 ± 80.9 vs 16.4 ± 16.6 IU/ml (p<0.001), HOMA IR 11.6 ± 24.6 vs 3.8 ± 4.8 (p<0.001), glucose 109 ± 84 vs. 90 ± 21 mg/dl (p<0.001), plasmogen activator inhibitor factor-1 (PAI-Fx) 28.8 ± 16.1 vs 14.5 ± 13.6 (p<0.001), and lower HDLC 43 ± 12 vs 50 ± 13 mg/dl (p<0.008). Significant independent explanatory variables for AST were interaction of HOMA IR * PAI-Fx (p<0.001), and for ALT, BMI (p<0.003), HOMA IR (p<0.001), and PAI-Fx (p<0.002). By logistic regression, determinants of combined high ALT and AST were PAI-Fx, OR 1.04, 95% CI 1.020-1.055, p<0.001 and HDLC, OR 0.94, 95% CI 0.902-0.976, p<0.002. Of 17 women with pretreatment ALT and AST both high, after 12.4 months on treatment, both ALT and AST normalized in 7 (41%), either normalized in 2 (12%), and both remained high in 8 (47%) vs 340 women with normal pretreatment ALT-AST, bilirubin after 12.6 months on therapy, 318 of whom (94%) retained normal levels, 15 (4%) had either ALT or AST and 7 (2%) had both, p<0.001.

In 18 women with both ALT and AST high pre-treatment, on treatment, insulin fell 43%, HOMA IR fell 69%, compared to 340 with normal ALT-AST and bilirubin whose insulin fell 30% and HOMA IR fell 11%, p<0.008.

Conclusions: In women with PCOS who have pretreatment high ALT and AST, on metformin-low GI diet, both ALT and AST normalized in 41%. Women with PCOS are likely to develop NAFLD, because of obesity, hyperinsulinemia, high PAI-Fx, hyperglycemia, and low HDLC, largely reduce factors with metformin-low GI diet.

ABSTRACT FINAL ID: 69

RAGE EXPRESSION IN HUMAN SKELETAL MUSCLE IS NORMALIZED FOLLOWING AEROBIC EXERCISE TRAINING IN OLDER OBSESE ADULTS

Abstract Body:
Advanced glycation endproducts (AGEs) mediate tissue damage and persisten inflammation through receptor independent and dependent interactions. The receptor for AGEs (RAGE) binds AGEs and other ligands, such as high mobility group box 1 (HMGB1), and induces a potent and cyclical cascade of pro-inflammatory events. RAGE expression has not been comprehensively examined in human skeletal muscle. Our purpose was to determine RAGE protein expression in human skeletal muscle and examine the effects of aerobic exercise training (ET) on RAGE protein expression and plasma AGEs and HMGB1 in obese insulin resistant subjects. Muscle biopsies were performed on 6 lean healthy controls (LHC: Age 37 ± 2, BMI 25 ± 1) and 18 obese insulin resistant subjects (OB: Age 66 ± 1, BMI 34 ± 1) prior to and following (OB-Post) a 12 week aerobic ET intervention (5d/wk, 60 min/d, 85% HRmax). Skeletal muscle protein (10 μg) was immunoblotted for RAGE and plasma was analyzed for carboxymethyllysine (CML) by liquid chromatography-tandem mass spectrometry and HMGB1 by ELISA. At baseline, OB subjects showed increased RAGE protein expression compared to the LHC group (LHC: 0.454 ± 0.046, OB: 0.572 ± 0.036, p<0.03). Following ET, RAGE protein expression was normalized to levels comparable to LHC (OB-Post: 0.454 ± 0.028, p=0.01), CML (ng/mL) was reduced by 68.55%, p=0.03). HMGB1 was unchanged with ET. Changes in skeletal muscle RAGE protein expression with ET were predicted by baseline RAGE expression (R=−0.81, p=0.001) and by fasting glucose (r=−0.53, p=0.02).

Changes in RAGE protein expression following ET were also associated with changes in plasma CML (r=0.65, p=0.01) and with changes in fasting glucose (r=−0.46, p=0.05). These data indicate that RAGE is highly regulated in skeletal muscle and that hyperglycemia may regulate RAGE expression in skeletal muscle. Further, aerobic ET was effective in normalizing RAGE protein expression and reducing circulating AGEs.
ABSTRACT FINAL ID: 71
FOXO1 IMPAIRS GLUCOSE TOLERANCE AND PROMOTES INSULIN RESISTANCE IN LIVER-SPECIFIC INSULIN RECEPTOR KNOCKOUT (LIRKO) MICE
I. O-Sullivan, W. Zhang, T. Unterman. Medicine, University of Illinois and Jesse Brown VA Medical Ctr. Chicago, II.; D. Beer-Stolz. University of Pittsburgh, Pittsburgh, PA; C. Kuhn, Joslin Diabetes Center, Boston, MA
Abstract Body:
FoxO proteins are important targets of insulin action and contribute to the regulation of gluconeogenic, glycolytic and lipogenic gene expression in the liver (JBC 281:10105, 2006). To better understand the role of FoxO proteins in mediating effects of insulin, we created liver-specific knockout mice targeting genes for FoxO proteins and/or the insulin receptor using the Cre-lox system. In initial studies, we examined effects of disrupting FoxO1 alone or FoxO1 in combination with FoxO3 and FoxO4 (FoxO1344) in liver. Disrupting FoxO1 alone had limited effects on glucose and lipid levels and glucose tolerance. In contrast, disruption of FoxO1, FoxO3 and FoxO4 together significantly improved glucose tolerance and increased circulating levels of triglycerides (Tg) and cholesterol, reflecting changes in expression of gluconeogenic (PEPCK, G6Pase), glycolytic (GK) and lipogenic (SREBP-1c) genes. Liver-specific insulin receptor knockout (LIRKO) mice had reduced body weight, liver/body weight ratio, serum Tg and cholesterol levels, and markedly improved glucose and insulin tolerated compared to floxed controls. Disruption of both IR and FoxO1 (LIRLOF) restored body weight, improved liver size, and reversed cellular dysglycemia, vacuolization and alterations in mitochondria seen in LIRKO liver. Deletion of IR plus FoxO1 also normalized glucose tolerance, improved expression of PEPCK, GK and SREBP-1c, and restored the ability of insulin to lower glucose levels despite the absence of hepatic insulin signaling. These results demonstrate a) multiple FoxO proteins contribute to the regulation of hepatic gene expression and metabolism, and b) regulation of FoxO1 in the liver plays a major role in maintaining glucose homeostasis and is critical to the ability of insulin to lower glucose levels through mechanisms that are independent of hepatic insulin signaling.

ABSTRACT FINAL ID: 73
SUCCESSFUL SWITCH FROM INSULIN TO SULFONYLUREAS IN KATP CHANNEL-RELATED NEONATAL DIABETES IS CORRELATED WITH YOUNGER AGE AT THE TIME OF TRANSITION
B.W. Thurber, J.T. Dickens, L.H. Philipson, R.N. Naylor, S.W. Greeley. Medicine, University of Chicago, Chicago, IL; A.N. Pastore. College of Medicine, University of Illinois at Chicago, Chicago, IL
Abstract Body:
Patients with neonatal diabetes due to heterozygous activating mutations of either gene (KCNJ11 or ABC8) encoding the two subunits of the KATP channel can usually be treated with oral sulfonylureas (SU) pills in lieu of insulin injections. However, data is limited on which factors may influence the degree of success with SU treatment even initially, much less so on the long-term. The purpose of this study was to test our hypothesis that younger age at the time of transition to SU therapy is correlated with success as gauged by Ha1c, SU dose, and requirement for additional diabetes medications. We utilized data from the University of Chicago Monogenic Diabetes Registry (http://monogenicdiabetes.uchicago.edu). Descriptive statistics and regression analysis were performed using Stata Version 12. After excluding those with transient neonatal diabetes or known unresponsive severe mutations from 71 total subjects with causal KCNJ11 mutations, reliable data on the success of treatment attempt was available on 30 cases. Overall, a significant decrease in Ha1c level was seen, from 8.1% before transition to 5.9% after SU therapy (P < 0.0001, n = 30). However, those who were older at the time of transition had less improvement in Ha1c and were more likely to continue to require some insulin and/or other diabetes medications. In particular, those who were 15 years of age or older were most likely to experience difficulty. Furthermore, linear regression showed a significant correlation between the age at transition and the dose (mg/kg/day) of sulfonyl urea required (R^2 = 0.59, n = 26, P = 0.003). While longer follow-up and more comprehensive data are needed, our data suggests that earlier age at initiation of SU treatment is predictive of success. As suggested by mouse models of KCNJ11-related diabetes, this may be due to loss over time of beta cell mass that may be better preserved by SU treatment. Our work supports the need for early genetic diagnosis and appropriate personalized treatment in all cases of neonatal diabetes.

ABSTRACT FINAL ID: 77
CONCORDANCE BETWEEN THE ESTIMATED HEMOGLOBIN A1C DERIVED FROM SHORT TERM CONTINUOUS GLUCOSE MONITORING SYSTEM MEAN GLUCOSE LEVEL AND THE MEASURED HEMOGLOBIN A1C IN SUBJECTS WITH DIABETES
V. Neagu, S. Ramirez, N. Chokshi, D. Mihaielisciu. University of IL at Chicago, Chicago, IL
Abstract Body:
Background: Hemoglobin A1c (HbA1c) is the most widely used test for estimating the degree of glycemic control in patients with diabetes, but it is unreliable if certain conditions that affect red blood cell survival, hemoglobin type and chronic kidney disease exist. Alternate methods for assessing long term glycemic control and guiding medical treatment for these patients are therefore needed. The aim of this study was to evaluate the concordance of estimated HbA1c (eHbA1c) derived from short term (72-96 hours) continuous glucose monitoring system (CGMS) mean glucose level, with the actual HbA1c level obtained before the CGMS study.
Methods: We retrospectively analyzed CGMS data of 37 patients with diabetes without any other known medical conditions that could affect HbA1c level. In order to be included in this study, patients had to have relatively stable glycemic control (without any changes in medical treatment or acute illnesses) for at least 3 months before the measurement of HbA1c and CGMS study. The eHbA1c was calculated from the formula described by Nathan et al. (eAG(mg/dL) = (28.7 x HbA1c) - 46.7) using the average sensor glucose level (eAG) obtained from the CGMS study. This value was compared with the serum HbA1c level obtained within 90 days prior to the CGMS test.
Results: Thirty-seven patients with diabetes (type one 27 (73%), female 21 (57%)) were included in this study. The mean eHbA1c was 7.1% compared to 8.4% for the mean measured HbA1c. Absolute differences between the two methods ranged between 0.1 and 3.2%, with 23 subjects (62%) having differences higher than 1%. The eHbA1c was consistently lower than the measured serum HbA1c level (P < 0.001 by one-tailed paired T-test). The Root Mean Square Error between the two tests was 1.57 indicating an insufficient concordance.
Conclusion: Based on our study results, eHbA1c derived from the mean CGMS glucose level cannot be used as a substitute for measuring HbA1c in patients with diabetes.

ABSTRACT FINAL ID: 81
OPTIMIZATION OF LDL CHOLESTEROL, TRIGLYCERIDES, HDL CHOLESTEROL AND AMELIORATION OF CARDIOVASCULAR EVENTS IN 190 PATIENTS, WHO HAD ≥5 YEARS FOLLOW-UP, AND MEDIAN AGE 84 YEARS AT LAST FOLLOW-UP
Abstract Body:
We retrospectively examined effects of treatment of low and high density lipoprotein cholesterol (LDLC, HDLC) and triglycerides (TG) on cardiovascular disease (CVD) in 190 patients (94 without, 96 with antecedent CVD), median age 71 years at study entry, who were ≥ age 80 at the end of follow-up. Our specific aim was to assess benefits of LDLC, HDLC and TG treatment in the elderly, focusing on prevention of new and repeat CVD events. We recorded lipids, BMI, morbid and lethal CVD events (MI, angina, angioplasty, coronary artery bypass graft [CABG], claudication, ischemic stroke, transient ischemic attack [TIAT]) at entry and during follow-up every
Subjects excluded or included from RCT

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Included in RCT: 61%+</th>
<th>Included in RCT: 61%+</th>
<th>Included in RCT: 61%+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>178</td>
<td>178</td>
<td>105</td>
</tr>
<tr>
<td>Contain, White [SO]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>59 (±1)</td>
<td>60 (±1)</td>
<td>60 (±1)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>33 (±2)</td>
<td>32 (±2)</td>
<td>32 (±2)</td>
</tr>
<tr>
<td>WC, cm</td>
<td>84 (±5)</td>
<td>84 (±5)</td>
<td>84 (±5)</td>
</tr>
<tr>
<td>WHR</td>
<td>0.84 (±0.04)</td>
<td>0.84 (±0.04)</td>
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</table>

Subjects included into RCT

midwestern regional meeting abstracts
ABSTRACT FINAL ID: 103
EFFECTS OF THE ENDOCRINE DISRUPTOR TOLYLFLUANID ON GLOBAL ENERGY METABOLISM
R.M. Sargis, E. El-Hashani, X. Zhang. Medicine, University of Chicago, Chicago, IL

Abstract Body:

The last several decades have witnessed a dramatic deterioration in global metabolic health with the burgeoning worldwide epidemic of obesity and diabetes. While consumption of a calorically-dense and highly palatable diet coupled with increased physical inactivity are central drivers of metabolic diseases, these factors alone fail to fully account for the magnitude and rapidity with which metabolic health has deteriorated. As such, increasing attention has turned to contributing factors that may potentiate the effects of lifestyle changes; this includes exposure to endocrine disrupting chemicals (EDCs), exogenous chemicals with the capacity to modulate endogenous hormonal and metabolic signaling pathways. Tolylfluanid (TF) is a phenylsulfamide fungicide used on fruit crops in agricultural regions outside of the United States as well as a booster biocide in marine paints to improve hydrodynamics. In previous work, this novel EDC was shown to promote proapoptotic-to-adipocyte differentiation in the 3T3-L1 cell line. TF also was shown to induce a state of insulin resistance in primary murine and human fat through a specific downstream regulation of the key insulin signaling intermediate, insulin receptor substrate-1 (IRS-1). The effects on adipose tissue are further accentuated by the binding of T3 to nuclear thyroid receptors (TR). Therefore, the maintenance of the proper intracellular T3 concentration is fundamental. For this, a concerted action of deiodinase activities and TH cell membrane transporters is important for the provision of optimal intracellular T3. While T3 is secreted by the thyroid gland, the brain, including the hypothalamus, maintains elevated serum thyrotropin concentrations despite high serum T3 levels. In contrast, the liver has normal T3 uptake, due to expression of T3T3. A LESSON FROM TWO CASES

Case 1: A 61-year-old male was admitted to the ICU with unexplained hypotension and fever. He was transferred from a nearby hospital following an episode of hypotension in the ED. The patient was not on any medications, had no history of cardiovascular disease, and was not on any beta blockers. On physical examination, he was afebrile, with a blood pressure of 80/60 mmHg, heart rate of 100 bpm, and respiratory rate of 20 breaths per minute. The patient was oriented to person but disoriented to place and time. He was hypoxic with an oxygen saturation of 90% on 4L/min of oxygen via nasal cannula. The patient was treated with intravenous fluids and empirical antibiotics, but his blood pressure remained low despite aggressive fluid resuscitation. The patient was transferred to the ICU for further management.

Second Case: A 60-year-old AA man with DM-2, obstructive sleep apnea (OSA), and HTN who arrived with septic shock, respiratory failure and anterior pituitary, D2 plays an important role in providing T3 to target cells. T4 and T3 are inactivated to rT3 and T2, respectively, by type 3-deiodinase (D3). In fetal life, D3 is expressed in placenta, uterus and in most fetal tissues, whereas brain is the main site in postnatal life.

The importance of transporters for TH action is best illustrated by the severe phenotype observed in mutations of the specific TH transporter MCT8 (monocarboxylate transporter 8) gene. Patients manifest a severe neurodevelopmental defect and abnormal distribution and metabolism of TH resulting in a thyroid phenotype, characterized by low serum levels of T4 and rT3 and high serum levels of T3.

ABSTRACT FINAL ID: 105
TRANSIENT PERINATAL HYPERTHYROIDISM IN MCT8 DEFICIENT MICE
A.M. Ferrara, X. Liao, R. Weiss, A.M. Dumitrescu, S. Refetoff. Medicine/Endocrinology, University of Chicago, Chicago, IL

Abstract Body:

The active thyroid hormones (TH), T3 and its precursor, T4 are essential for the normal development of mammals. Most TH actions result from classical TH-mediated gene expression. T3 is transported into target tissues through the 5'-deiodination of T4 catalyzed by the type 2 deiodinase (D2). In brain, developing cochlea, brown adipose tissue, and target tissues through the 5'-deiodination of T4 catalyzed by the type 2 deiodinase (D2). In fetal life, T3 is expressed in placenta, uterus, and in most fetal tissues, whereas brain is the main site in postnatal life.

The importance of transporters for TH action is best illustrated by the severe phenotype observed in mutations of the specific TH transporter MCT8 (monocarboxylate transporter 8) gene. Patients manifest a severe neurodevelopmental defect and abnormal distribution and metabolism of TH resulting in a thyroid phenotype, characterized by low serum levels of T4 and rT3 and high serum levels of T3.

Mct8KO mice faithfully replicate the TH changes observed in humans with MCT8 gene mutations even though they lack the severe neurologic defects. The animal model has provided a valuable tool to advance our understanding of the mechanisms that determine the thyroid phenotype in MCT8 deficiency. Brains of adult Mct8KO mice show low T3 uptake, decreased T3 content, increased D2 and decreased D3 activities as a consequence of impaired intracellular TH transport. The resulting TH depletion in brain produces a local hypothyroidism. The impairment of TH entry into the brain, including the hypothalamus, maintains elevated serum thyrotropin concentrations despite high serum T3 levels. In contrast, the liver has normal T3 uptake, due to expression of other transporters. In addition, the high D1 activity and the increased T3 content in liver reflect a state of local hyperthyroidism.

Most information on the pathophysiology of the Mct8 deficiency syndrome has been derived from studies on adult and late postnatal mice. However, there are no reports on the thyroid function during perinatal period or at birth. It is unclear whether the adult thyroid phenotype of Mct8KO mice is a continuation of the fetal levels or whether there are unique adaptations to early life in the absence of the Mct8. Treatment of the severe neurocognitive defect in humans would require precise information on the ontogeny of thyroid function allowing for early fetal intervention.

In this study, we provide an analysis of the thyroid phenotype and TH action during the immediate perinatal period of Mct8KO mice. We first evaluated the thyroid function tests of both Mct8KO and wild-type (Wt) mice from embryonic day 17 (E17) to postnatal day 7 (P7). Unexpectedly, Mct8KO mice showed high T4 at ages E18 and P0, the day prior to birth and the first day of life, respectively. We studied how this early T4 excess affected the TH metabolism and action in two TH sensitive tissues: the cerebral cortex and the liver. The results indicated that high serum T4 levels are accompanied by an enhanced TH action in cortex at E18 and P0, and in liver at P0. Both tissues, moreover, showed changes in the expression profiles of “alternative” TH transporters.

ABSTRACT FINAL ID: 107
UNEXPLAINED HYPOTENSION IN THE INTENSIVE CARE UNIT: EUTHYROID SICK OR SICK HYPOTHYROID – A LESSON FROM TWO CASES
K. Gharibbe, C.A. Koch, R. Ayusula, T. Fülöp. University of Mississippi Medical Center, Jackson, MS; É. Csongrádi. University of Debrecen, Debrecen, HUNGARY

Case Report Body:

Unexplained hypotension in the intensive care unit (ICU) setting is commonly attributed to volume depletion, cardiac failure, sepsis and/or relative adrenal insufficiency. Hypothyroidism usually associated with hypertension in non-ICU patients is considered less frequently in the differential diagnosis. As interpretation of thyroid testing results is more challenging in the critically ill patients, diagnosis may be delayed leading to the potential harm to these patients.

First Case: A 71-year-old African American (AA) female with stage 4 CKD, hypertension (HTN) and type-2 diabetes mellitus (DM-2) was admitted for urosepsis, Bradycardia and hypothermia. She responded initially well to IV fluids, antibiotics and empirical steroids but experienced persistently suppressed serum TSH, free T4, and T3. Thyroid function testing returned with only modest elevation of Thyroid Stimulating Hormone (TSH) of 7.65 µIU/mL [0.27–4.20], but free T4 was low at 0.66 ng/dL [0.93–1.70]. After a trial of IV levothyroxine (100 mcg/day), blood pressure (BP) rapidly rose, mental status improved and she was weaned from the respirator with ease. Further clinical follow-up ruled out central hypothyroidism.

Second Case: A 60-year-old AA man with DM-2, obstructive sleep apnea (OSA), and HTN who arrived with septic shock, respiratory failure and
renal failure secondary to S. hemolyticus bacteremia. He maintained a fair urine output despite being dependent on norepinephrine to maintain BP. TSH proved rapidly and was extubated rapidly thereafter.

**Discussion and Conclusion:** TSH is commonly suppressed in otherwise euthyroid but critically ill patients. Therefore, seemingly “normal” or only mildly abnormal TSH values in a critically ill ICU patient are worrisome for a potential hypothyroid state. The usual threshold for abnormal laboratory values in diagnosing myxedema comatosa may not apply under such circumstances; the concept of “thyroid insufficient state” may better describe these circumstances. The presence of co-morbid OSA may represent a further barrier hindering successful weaning from mechanical ventilation. Early reattribution of hypothyroidism-induced or further aggravated existing cardioimpoty. The concept of relative thyroid insufficiency in the ICU setting needs additional studies and exemplifies individualized medicine.

**GASTROENTEROLOGY/ClinICAL NUTRITION ABSTRACT FINAL ID: 63**

**EPIDEMIOLOGY OF ACUTE PANCREATITIS IN HOSPITALIZED CHILDREN IN THE UNITED STATES FROM 2000-2009**

C. Pant, M.P. Anderson. Pediatric Gastroenterology; University of OK Health Sciences Center, Oklahoma City, OK; T.J. Sferra. Pediatric Gastroenterology; Case Western University, Cleveland, OH; A. Deshpande. Infectious Diseases, Case Western University, Cleveland, OH

**Abstract Body:**

**Objective:** Recent single-center studies suggest an increasing incidence of acute pancreatitis (AP) in children. We sought to estimate the secular trends and outcomes of AP in hospitalized children in the United States.

**Methods:** We used the U.S. Healthcare Cost and Utilization Project Kids’ Inpatient Database. Data were weighted to generate national-level estimates.

**Results:** We identified 55,012 cases of AP during four triennial periods from 2000 to 2009. The rate of hospitalization (incidence) of children with acute pancreatitis (AP) in children. We sought to estimate the secular trends and outcomes of AP in hospitalized children in the United States. Despite the improvement in mortality and LOS during recent years, hospitalized children with AP have worse outcomes than those without this disease.

**GENETIC & MOLECULAR MEDICINE ABSTRACT FINAL ID: 7**

**A BARRIER TO DIFFUSION OF OPSINS BUT NOT PERIPHERAL MEMBRANE PROTEINS AT THE CONNECTING CILIUM AND DISK RIMS OF CONE PHOTORECEPTOR SENSORY CILIA**

L.I. Geneva, C.M. Ignacio, B.E. Knox, P.D. Calvert. SUNY Upstate Medical University, Syracuse, NY

**Abstract Body:**

**Purpose:** To reveal the mechanisms allowing signaling proteins to be concentrated within the outer segment (OS) of cone photoreceptors. Localization and retention of transduction cascade proteins within ciliary signaling compartments are thought to be mediated, in part, by diffusion barriers at the transition zone. In the retina, mutations in cone opsins and other phototransduction cascade proteins often result in their mislocalization from the ciliary cone OS, and can lead to a spectrum of diseases, ranging from color blindness to progressive photoreceptor dystrophy, and eventually blindness.
While the mechanisms for protein transport to and retention within the rod OS have received considerable attention, little is known about these processes in cones. Unlike rods, the OS discs of cones are contiguous with the plasma membrane, and therefore retention of opsin would require a diffusion barrier either at the cilium base, as has been demonstrated for primary cilia, or at the disc rim. To address this problem, we examine the diffusion of the transmembrane red cone opsin-EGFP fusion protein and the peripheral membrane protein double geranylgeranyl-EGFP expressed in cones of Xenopus laevis using multiphoton FRAP.

Methods: Transgenic X. laevis tadpoles were created using the REMI method to express either red opsin-EGFP or double geranylgeranyl-EGFP exclusively in their cones. Confocal and multiphoton imaging of living cones from isolated retinas cut in chips were used to study the localization and dynamics upon photobleaching of these proteins. Custom built MATLAB routines were used for quantification of the collected images.

Results: First, we show that both proteins diffuse laterally in the disc membranes with similar rates, diffusion of opsin-EGFP between discs is significantly retarded compared with double geranylgeranyl-EGFP and therefore axial equilibration for opsin is delayed. Second, we show that while double geranylgeranyl-EGFP has access to all cone membranes, opsin is excluded from all cone segments except for the OS where it is highly concentrated.

Conclusions: We identified a selective barrier to free diffusion for coneopsin at the level of the connecting cilium as well as impediment to diffusion at the end loops between adjacent discs where the latter does not impede the free movement of peripheral membrane proteins.

ABSTRACT FINAL ID: 37

**TGFβ SIGNALING INDUCES EXPRESSION OF GADD45B IN RETINAL GANGLION CELLS**

B. Liu, X. Sun, J.G. Garcia, Medicine, University of IL, Chicago, IL; B. Liu, Y.J. Leiderman. Ophthalmology, University of IL, Chicago, IL; G. Saeyoka. Ophthalmology, Northwestern University, Chicago, IL

**Abstract Body:**

**Rationale:** Growth arrest and DNA damage protein 45b (Gadd45b) functions as an intrinsic neuroprotective molecule protecting retinal ganglion cells (RGCs) from injury. This study was performed to elucidate further the induction pathway of Gadd45b expression in RGCs.

**Methods:** The induction of Gadd45b expression in response to TGFβ1/NFκB signaling was investigated in RGC5 cultures in vitro and murine retina in vivo. Gadd45b mRNA and protein expression were detected by quantitative real-time RT-PCR, immunoblot assay, immunohistochemistry, and immunocytochemistry. Activation of NFκB and TGFβ/Gadd45b signaling were assessed by measuring phosphorylation of NFκB and using specific inhibitors. Gadd45b siRNA was transfected into RGC5 to silence Gadd45b mRNA expression. Expression of TGFβ1 receptors I and II was detected in RGCs in vitro and RGCs in vivo. TGFβ1 induced abundant Gadd45b mRNA and protein expression, exhibiting a dose-dependent response in vitro. Exogenous TGFβ1 induced up-regulation of Gadd45b expression in RGCs in murine retina in vivo. TGFβ1 stimulated phosphorylation of NFκB, and inhibition of NFκB phosphorylation blocked induction of Gadd45b by TGFβ1 in RGC5. Induction of Gadd45b by TGFβ1 increased the resistance of RGC5 against TNFα cytotoxicity and paraxial oxidative stress.

**Conclusions:** TGFβ signaling induced Gadd45b expression in RGCs. Modulation of the TGFβ1/NFκB/Gadd45b signaling pathway may provide a means to enhance the neuroprotective effect of Gadd45b in RGCs.

**ABSTRACT FINAL ID: 45**

**LONG-TERM USE OF COCHLEAR IMPLANTS IN OLDER ADULTS: RESULTS FROM A LARGE CONSECUTIVE CASE SERIES**

J.S. Choi, K.J. Contrera. Johns Hopkins School of Medicine, Baltimore, MD; C. Blake, I.K. Niparko, F.R. Lin. Otolaryngology-Head & Neck Surgery, Johns Hopkins School of Medicine, Baltimore, MD; J. Betz. Biostatistics, Johns Hopkins School of Medicine, Baltimore, MD; F.R. Lin. Epidemiology, Johns Hopkins School of Medicine, Baltimore, MD

**Abstract Body:**

**Introduction:** A cochlear implant (CI) is a surgically-implanted neuromusculoskeletal device that allows for access to sound for individuals with severe-to-profound sensorineural hearing loss. Previous studies have shown that older adults consistently demonstrate improved speech perception abilities after cochlear implantation, but whether older adults continue to utilize CI over the long term is unknown. We investigated rates of long-term use of cochlear implants in a large, consecutive case series of older adults (> 60 years).

**Methods:** From 1999-2011, 447 individuals ≥ 60 years received their first CI at Johns Hopkins, and we successfully contacted 397 individuals (89%) via email, phone, and postal survey to ascertain data on the individual’s daily CI use averaged over the past 4 weeks. Regular CI use was defined as ≥ 8 hours/day. We investigated the time from implantation to the date when an individual discontinued regular CI use with Kaplan-Meier and Cox proportional hazard analyses.

**Results:** The overall rate of regular CI use was 93.2% [95% Confidence Interval: 89.9-95.4%] at 5 years of follow-up and 82.6% [95% CI: 72.5-89.3%] at 13.5 years. Individuals who received a CI at 60-74 years had significantly higher rates of regular CI use (91.1%, [95% CI: 83.2-95.4%]) than individuals who received a CI at ≥ 75 years (55.7%, [95% CI: 24.9-78.1%]). The risk of discontinuing regular CI use (< 8 hours per day) was linearly associated with the age at implantation (increased risk of 7.8% [95% CI: 3.0-12.8%] per year of age at implantation). For patients reporting less than 8 hours of use per day, the most common causes were poor hearing benefit (45%), pain or discomfort (23%), no need to hear (23%), comorbid illnesses (19%), and device failure (7%).

**Conclusion:** Rates of long-term CI use in older adults ≥ 10 years of follow-up exceed 80% with the risk of discontinuing regular CI use being strongly associated with greater age at implantation. These results suggest that the earlier implantation of older adults may be associated with better outcomes.

**HEMATOLOGY AND ONCOLOGY**

**ABSTRACT FINAL ID: 3**

**DOWNREGULATION OF JCV T-ANTIGEN BY HYPOXIA AND GLUCOSE DEPRIVATION IN MEDULLOBLASTOMA**

E. Noch, I.K. Sariyer, J. Gordon, K. Khalili. Neuroscience, Temple University School of Medicine, Philadelphia, PA

**Abstract Body:**

Recent studies have reported the detection of the human neurotropic virus, JCV, in a significant population of brain tumors, including medulloblastomas. Expression of the JCV early protein, T-antigen, which has transforming activity in cell culture and in transgenic mice, results in the development of a broad range of tumors of neural crest and glial origin. Evidently, the association of T-antigen with a range of tumor-suppressor proteins, including p53 and pRb, and signaling molecules, such as β-catenin and IRS-1, plays a role in the oncogenic function of JCV T-antigen. We demonstrate that T-antigen expression is suppressed by hypoxia and glucose deprivation in medulloblastoma cells and in glioblastoma xenografts that both endogenously express T-antigen. Mechanistic studies indicate that hypoxia-mediated T-antigen downregulation is due to ubiquitin-mediated degradation and that glucose deprivation-mediated suppression of T-antigen is partially influenced by 5′-activated AMP kinase (AMPK), an important sensor of the AMP:ATP ratio in cells. In addition, glucose deprivation-induced cell cycle arrest in the G1 phase is blocked with AMPK inhibition, which also prevents T-antigen downregulation. Furthermore, T-antigen prevents G1 arrest and sustains cells in the G2 phase during glucose deprivation. On a functional level, T-antigen downregulation is partially dependent on reactive oxygen species (ROS) production glucose deprivation, and T-antigen prevents ROS induction, loss of ATP production, and cytotoxicity induced by glucose deprivation. We have also found that T-antigen is downregulated by the glycolytic inhibitor, 2-deoxy-D-glucose (2-DG), and the pentose phosphate inhibitors, 6-aminonicotinamide and oxythiamine, and that T-antigen modulates expression of the glycolytic enzyme, hexokinase 2 (HK2), and the pentose phosphate enzyme, transaldolase-1 (TALDO1), indicating a potential link between T-antigen and metabolic regulation. These studies point to the possible involvement of JCV T-antigen in medulloblastoma proliferation and the metabolic phenotype and may enhance our understanding of the role of viral
proteins in glycolytic tumor metabolism, thus providing useful targets for the treatment of virus-induced tumors.

ABSTRACT FINAL ID: 5
KINETOCHEORE-MICROTUBULES MEDIATE RADIATION-INDUCED GENOME DAMAGE
Abstract Body:
The exquisite sensitivity of mitotic cancer cells to ionizing radiation underlies the rationale for fractionated radiation therapy. Nonetheless, the mechanism of this cell cycle-dependent vulnerability is unknown. Here we show that ionizing radiation selectively increases the stability of microtubule attachments to chromosomes at kinetochores, eliciting a dose-dependent surge of kinetochrome-microtubule attachment errors during chromosome segregation. These errors, manifested by lagging chromosomes in anaphase, generate long-term aneuploidy, a preponderance of micronuclei, and chromosomal pulverization. Destabilizing, or temporarily abolishing, microtubule attachments to chromosomes leads to reduction of these defects, substantial increase in the viability of irradiated mitotic cells, and radiation resistance in orthotopically transplanted human glioblastoma multiforme tumors. Alternatively, pharmacologically increasing kinetochrome-microtubule attachment errors potentiates radiation-induced genome damage. Thus, kinetochrome-microtubules represent prominent cellular targets of ionizing radiation that lead to widespread genome damage beyond direct DNA breaks and they offer additional means to sensitize tumors to radiation therapy. Finally, to emphasize the clinical relevance of these findings, we show that increased rates of lagging chromosomes in rectal adenocarcinoma substantiates an enhanced response to chemo-radiation therapy thereby supporting the role of whole-chromosome mis-segregation in mitigating radiation-induced damage.

ABSTRACT FINAL ID: 83
DEEP VENOUS THROMBOSIS, PULMONARY EMBOLISM, AND OSTEONECROSIS AFTER STARTING EXOGENOUS TESTOSTERONE THERAPY IN MEN AND WOMEN WITH PREVIOUSLY UNDIAGNOSED THROMBOPHILIA OR HYPOFIBRINOLYSIS
Abstract Body:
Objective: Our specific aim was to describe thrombosis (deep venous thrombosis [DVT]-pulmonary embolism [PE], and osteonecrosis [ON]) of the hips-knees that developed after testosterone (T) therapy in 12 previously healthy Caucasians (11 men, 1 woman) with no antecedent thrombosis and previously undiagnosed thrombophilia-hypofibrinolysis. Materials/Methods: Patients sustaining thrombotic events (DVT-PE) or ON while taking T were evaluated by studies of familial and acquired thrombophilia and hypofibrinolysis. Results: Of the 7 men who developed DVT-PE on T therapy, 2 were found to be heterozygous for the Factor V Leiden mutation, 5 had high Factor VIII, 1 had high Factor XI, and 1 had low antithrombin III. Of the 4 men and 1 woman who developed ON on T, 1 was heterozygous for the Factor V Leiden mutation, 2 had plasminogen activator inhibitor gene 4G4G homozygosity, 1 had high anticardiolipin antibody IgG, and 1 had no clotting abnormalities. In 3 thrombophilic men with DVT-PE, when T was continued after the first thrombotic event, despite therapeutic INR on warfarin, DVT-PE recurred. Conclusions: DVT-PE and ON after starting T are associated with previously undiagnosed thrombophilia-hypofibrinolysis. We speculate that when exogenous T is aromatized to E2, and E2-induced thrombophilia is superimposed on thrombophilia-hypofibrinolysis, thrombosis occurs. Men and women sustaining thrombotic events on T therapy should be screened for familial and acquired thrombophilias, and subsequent exogenous T is contraindicated in the presence of thrombophilia.

ABSTRACT FINAL ID: 49
MORTALITY FROM METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS BACTEREMIA: META-ANALYSIS
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Abstract Body:
Purpose of the study: Methicillin-resistant Staphylococcus aureus (MRSA) bacteremia is thought to be associated with a significantly higher mortality rate than methicillin-sensitive Staphylococcus aureus (MSSA) bacteremia. However, because of the changing epidemiology and advances in infection control measures the association remains controversial. In this meta-analysis we evaluated the impact of methicillin resistance on mortality in MRSA bacteremia. Methods: We searched MEDLINE and 3 other electronic databases for subject headings and text words related to MRSA/MSSA bacteremia and mortality in articles published from 1966 to 2012. Only multivariate studies that found MRSA to be an independent predictor of mortality were assessed. Data was independently extracted by 2 authors. Individual weights and Odds ratios (OR) were calculated using the 95% CI using the random-effects model. Results: Twenty-eight studies (n=11,625 patients) met the inclusion criteria. Patients with MRSA bacteremia were two times more likely to die than those with MSSA bacteremia (summary OR: 1.74; 95% CI, 1.52-1.98, P<0.00001, I 2 = 21%). Subgroup analyses, in which adjustments for confounding variables were made, revealed a consistent increase in mortality in patients with MRSA bacteremia. Conclusions: Methicillin resistance continues to be associated with increased mortality among patients with Staphylococcus aureus bloodstream infection.

ABSTRACT FINAL ID: 65
WITHIN THE BROAD RANGE OF NORMAL RENAL FUNCTION, SERUM CREATININE IS AN INDEPENDENT PREDICTOR OF SERUM HOMOCYSTEINE LEVELS
C.M. Richardson-Royer, S. Munsif, G. Boriel, M. Gowda, J. Padda, C.J. Glueck. Internal Medicine, Jewish Hospital, Cincinnati, OH; C.M. Richardson-Royer, S. Munsif, G. Boriel, M. Gowda, J. Padda, P. Wang, C.J. Glueck. Jewish Hospital Cholesterol and Metabolism Center, Cincinnati, OH
Abstract Body:
Background: Serum homocysteine levels are biomarkers for subclinical target organ damage and, to a large degree, depend on renal function. Our specific aim was to assess whether, and to what degree, serum creatinine and estimated glomerular filtration rate (eGFR) were determinants of serum homocysteine in patients selected by normal serum creatinine and eGFR. Methods: We performed a retrospective clinical case series study of determinants of serum homocysteine in 1000 patients (489 women, 511 men) consecutively referred for diagnosis and treatment of hyperlipidemia, and selected by normal renal function (serum creatinine $\leq 1.27$mg/dl in men, $\leq 1$ mg/dl in women) and normal eGFR ($\geq 59$ ml/min/1.73m$^2$). Results: Serum Homocysteine was positively correlated with serum creatinine ($r=0.25$, $p<0.0001$) and was inversely correlated with eGFR ($r=-0.17$, $p<0.0001$).
p < .0001). By stepwise multiple regression with homocysteine as the dependent variable and eGFR, creatinine, methylmalonic acid, triglyceride, HDL cholesterol, age, sex, and race as explanatory variables, we accounted for 73.3% of the variance of serum homocysteine. The most significant explanatory variables were creatinine (partial R2 = 3.5%, p < .0001), methylmalonic acid (partial R2 = 3.0%, p = .0001), and age (partial R2 = 0.8%, p = .006).

**Conclusion:** Within the broad range of normal renal function as assessed by creatinine and eGFR, higher creatinine and lower eGFR were associated with higher serum homocysteine and thus higher risk for cardiovascular disease (CVD). Optimization of renal function, even within the normal range, particularly in prehypertensive subjects, may be important in prevention of CVD mediated by homocysteine.

**ABSTRACT FINAL ID: 75**

**STATINS DO NOT MITIGATE RADIATION NEPHROPATHY**

E.P. Cohen. Medicine, Medical College Wisconsin, Milwaukee, WI; B.L. Fish, J.E. Moulder. Radiation Oncology, Medical College Wisconsin, Milwaukee, WI

**Abstract Body:** Angiotensin-converting enzyme inhibitors (ACEi) mitigate radiation nephropathy (Rad np) caused by single or fractionated irradiation. But ACEi only mitigate, and do not cure Rad np. We tested statins to achieve additional benefit.

We tested atorvastatin, simvastatin and pravastatin in our rat total body irradiation (TBI) plus bone marrow transplant (BMT) model. Both male and female 8-week-old WAG/Rij/Cmcr rats were studied.

**Atorvastatin:** 36 male rats received 10 Gy in single fraction followed by a BMT. 10 rats were given TBI alone, 5 were given TBI plus atorvastatin 500 mg/kg in the diet (40 mg/kg/day) starting 8 days after TBI. 10 rats were given TBI + the ACEi captopril 150 mg/L in the drinking water (13 mg/kg/day) and 11 rats were given TBI + the combination of both drugs. Rats were followed for renal function and survival. In our model, rats that reach a BUN > 120 mg/dl or that are unwell from renal failure must be sacrificed. Renal function and survival were the same in the TBI alone compared to TBI + atorvastatin group. Rats undergoing TBI + captopril or TBI + the combination had significantly better renal function and survival compared to TBI alone. The TBI + combination showed no additional benefit when compared to the TBI + captopril group.

**Simvastatin:** 32 female rats received 10 Gy in single fraction followed by a BMT. 8 rats were given TBI alone, 8 were given TBI plus simvastatin 120 mg/kg in the diet (10 mg/kg/day) starting 9 days after TBI. 8 rats were given TBI + the ACEi inhibitor captopril 300 mg/L in the drinking water (34 mg/kg/day) and 8 rats were given TBI + the combination of both drugs. Rats were followed for renal function and survival. Renal function and survival were the same in the TBI alone compared to TBI + atorvastatin group. Rats undergoing TBI + captopril or TBI + the combination had significantly better renal function and survival compared to TBI alone. The TBI + combination showed no additional benefit when compared to the TBI + captopril group.

**Pravastatin:** 28 female rats received 19 Gy in 6 fractions over 3 days followed by a BMT. 9 rats had pravastatin 35 mg/L in the drinking water (4 mg/kg/day) starting right after BMT. Rats were sacrificed 9 weeks after TBI. Rats undergoing irradiation without drug and irradiated rats on pravastatin developed azotemia that did not differ between groups. Serum from control and irradiated rats receiving statins were tested for cholesterol and triglyceride levels. Rats receiving statins had lower lipid levels than rats not receiving drug, indicating that they were getting a biologically effective dose. Despite this, statins neither mitigate radiation nephropathy when given alone, nor do they add to the benefit of the ACEi captopril.


**ABSTRACT FINAL ID: 109**

**RECALCITRANT, MULTIPLE ANTIBIOTICS-RESISTANT STAPHYLOCOCCUS EPIDERMIDIS BACTEREMIA RESOLVING UPON LIGATION OF ARTERIO-VENOUS HEMODIALYSIS GRAFT IN AN END-STAGE RENAL DISEASE PATIENT**

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**Case Report Body:** A 53-year-old African-American male presented with worsening dyspnea and disproportionate left upper extremity edema. He had two similar admissions during the last one month before for volume overload, treated on both occasions with aggressive daily dialysis and fluid removal only. There was no history of fever or constitutional signs. His past medical history included end-stage renal disease on hemodialysis via a left upper extremity arterio-venous graft (AVG), type-2 diabetes mellitus, high blood pressure and obstructive sleep apnea. A new AVG was already in place and maturing on the right upper extremity.

Physical exam revealed diffuse edemas with disproportionate severe swelling of the left arm. Vital signs with temperature 36.5 C, pulse rate 69 (beats/minute), blood pressure with 124/64 mmHg, respiratory rate 18/min with 02 saturation of 95% while breathing room air. Chest exam noted diffuse bibasilar rales but clear lung fields on X-ray. CT angiogram during previous admission was negative for pulmonary embolism, but described bilateral ground glass opacities and borderline lymphadenopathy. Ultra-sound and AV graft ultrasound studies confirmed occlusion of the left subclavian vein with marked collateral vessels draining into the superior vena cava. Surveillance blood cultures (BC) grew Gram positive cocci (methicillin-resistant Staphylococcus epidemidis) repeatedly. Despite multiple antibiotic therapy (vancomycin, rifampin and gentamicin) BC remained persistently positive (six sets of BC in total). Cardiac echocardiogram remained normal (vee for vegetations. Interestingly, he remained free of any clinical signs of infection throughout the hospital course. White blood cell count remained normal: 4,600 -6,000/mm3. Biomarkers of inflammation noted only marginal elevation of procalcitonin: 0.6 and 1.06 mg/mL [0.05] and repeatedly unremarkable C-reactive protein values: 0.2 mg/dl [0.49]. A decision was made to ligate the left upper arm AVG, after which not only arm swelling resolved rapidly but also systemic bacteremia ceased.

**Conclusion:** In our patient with presumed infected AVG, disruption of genetic flow through the AVG plus function of the AVG was postulated as a possible source of bacteremia. Occult bacteremia should enter the differential of unexplained third-spacing/edema formation in a dialysis patient with implanted artificial material. High-output heart failure due to excessive arterio-venous shunting (with both upper arms with functioning AVG in place) represented an alternative explanation for systemic edema formation but not for the antibiotic-resistant bacteremia.
Migration assays indicate that MYLKP increases cell migration in H23 and Beas2b cells. SNP association study analysis identified two SNPs in the MYLKP promoter that are associated with colon cancer in African American patients. Luciferase constructs were designed containing the MYLKP promoter with each genotype combination of the two candidate SNPs. Luciferase assays showed increased promoter activity when both minor alleles are present. In silico analysis of the SNPs showed alterations in the binding sites for Pax9, AML1, TLX1, and ZNF300, which suggests possible mechanisms for the alteration in promoter activity.

**Conclusion:** In vitro data confirm that MYLKP contributes to oncogenesis and that two MYLKP promoter SNPs are associated with colon cancer. Further, these SNPs alter the activity of the MYLKP promoter and alter the binding sites for four transcription factors. Ongoing studies aim to confirm the in silico data and determine a mechanism of MYLKP function. We anticipate that these studies could aid in development of personalized chemotherapeutics for lung and colon cancer patients.

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**ABSTRACT FINAL ID: 19**

**SEPsis INDUCes Acute EMPHYSEMA in A G-PROTEIN COUPLED LPA2 DEFICIENT MICE**

J. Zhao, Y. Zhao, R.K. Mialki, J. Wei, A.S. Leme, S.D. Shapiro. Department of Medicine, University of Pittsburgh, Pittsburgh, PA; P. Fu, L. Huang, V. Natarajan. University of IL at Chicago, Chicago, IL.

**Abstract Body:**

**Rationale:** Lysophosphatidic acid (LPA) plays a dual-function in lung inflammatory diseases. LPA receptor is a member of G-protein-coupled receptor (GPCR) family which contributes to the pathogenesis of asthma, acute lung injury, and fibrosis. However, the pathogenesis role of LPA receptor in sepsis-induced lung inflammation and injury. **Methods and Results:** A murine model of mild sepsis was induced using cecal ligation and single puncture (CLP) with 27 gauge needle. After 24 h, plasma, bronchoalveolar lavage (BAL), and lung tissues were collected. Septic LPA2-/- mice reduces plasma KC, not IL-6, compared to the septic wild type mice. BAL KC increased in septic wild type and LPA2-/- mice, while there was no significant difference between these two groups. Mild sepsis had no effect on BAL IL-6 levels, protein leak, and inflammatory cell infiltration in the lungs in all sham and septic wild type and LPA2-/- mice. Interestingly, Hematoxylin and eosin (H&E) staining revealed that unlike wild type mice, septic LPA2-/- mice aggravated alveolar space enlargement ~1.57 fold, compared to sham LPA2-/ mice. Western blotting analysis of lung tissues demonstrated that cortactin, a F-actin binding protein, was decreased in septic LPA2-/ mice, when compared to wild type mice. The level of immunoglobulin G (IgG) in BAL fluids significantly increased in septic LPA2-/ mice, compared to septic wild type mice and sham mice. Furthermore, we found that sham and septic LPA2-/- mice increased surfactant proteins B, C, and D (SP-B, SP-C, and SP-D) expression in lungs, while SP-A levels in lungs was decreased in sham and septic LPA2-/- mice.

**Conclusions:** These results suggest the GPCR receptor LPA2 may regulate cortactin and surfactant protein expression in the lung. Mild sepsis induced alveolar space enlargement in LPA2-/ mice. LPA2 and its downstream signaling may play a protective role against sepsis-induced emphysema like disease.

The study is supported by NIH ROI HL091916 (to YZ) and American Heart Association Science Developmental Grant (to IJ)

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**ABSTRACT FINAL ID: 21**

**F-BOX PROTEIN DEGRADATION CASCADE REGULATES LUNG EPITHELIA CELL MIGRATION**


**Abstract Body:**

The ubiquitin-proteasome pathway is the major system for protein degradation. The Skp-Cull-F-box protein (SCF) ligase complex is one of the largest E3 ubiquitin ligase families. The F-box protein in the E3 ligase complex functions as the bridge by linking the ligase complex and specific substrates via its F-box domain and substrate binding motif. However, F-box protein stability has not been well studied. Here, we show that a relatively new F-box protein, termed FBXL19, is an unstable protein and degraded in another F-box protein (FBXW17)-mediated proteasome system. The cycloheximide chase assay showed that FBXL19 is a polyubiquitinated protein with a half-life ~ 2h. The proteasome inhibitor (MG-132), not lysosome inhibitor (leupeptin), attenuated FBXL19 degradation. We identified that another F-box protein, termed FBXW17 targets FBXL19 for ubiquitination and degradation. Over-expression of FBXW17 induced FBXL19 degradation, while knock-down of FBXW17 increased FBXL19 stability. By screening a series of lysine to arginine mutants of FBXL19, we found that lysine114 with FBXL19 is the ubiquitin acceptor site. The FBX19K14A mutant was resistant to FBXW17-mediated ubiquitination and degradation. Further, the new biological function of FBX19 was uncovered. Over-expression of FBX19 wild type or FBX19K14A mutant reduced serum- or lysophosphatidic acid (LPA)-mediated cell migration, while the inhibitory effects of FBX19 wild type, but not of FBX19K14A mutant, were attenuated by over-expressed FBXW17. This is the first report to demonstrate that the SCF E3 ligase complex F-box protein targets another family member for ubiquitin-proteasomal degradation and that an F-box protein cascape regulates cell migration.

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**ABSTRACT FINAL ID: 23**

**FUNCTIONAL ANALYSIS OF MYOSIN LIGHT CHAIN KINASE GENE PROMOTER AND INFLAMMATORY LUNG DISEASE ASSOCIATED POLYMORPHISMS**


**Abstract Body:**

**Rationale:** Myosin light chain kinase (MLCK), a central cytoskeletal regulator encoded by MYLK gene, regulates muscle contraction, cell migration, endothelial cell-cell adhesion, and barrier function, thereby playing key pathophysiological roles in lung inflammatory diseases. We previously identified that MYLK single nucleotide polymorphisms (SNPs) as well as haplotypes are strongly associated with severe sepsis, acute lung injury and asthma in African Americans (AA) and European Americans (EA). Here we examined genetic and epigenetic regulation of MYLK promoter as well as the effects of SNPs on MLCK expression and activity, thereby influencing cytoskeletal balance and cell integrity.

**Methods:** In silico analysis of MYLK 5’UTR was performed. A series of 5’UTR deletions from the ~ 2.5 kb putative promoter fragment were fused to luciferase reporter vectors, and transfected into human lung endothelium. The DNA fragments containing SNPs were generated by site-mutagenesis. Transfection experiments were performed with luciferase reporter encoded by MYLK gene, regulates muscle contraction, cell migration, endothelial cell-cell adhesion, and barrier function, thereby playing key pathophysiological roles in lung inflammatory diseases. We previously identified that MYLK single nucleotide polymorphisms (SNPs) as well as haplotypes are strongly associated with severe sepsis, acute lung injury and asthma in African Americans (AA) and European Americans (EA). Here we examined genetic and epigenetic regulation of MYLK promoter as well as the effects of SNPs on MLCK expression and activity, thereby influencing cytoskeletal balance and cell integrity.

**Results:** MYLK promoter for mmMLCK contains distal inhibitory regulator and proximal enhancing regulator binding regions. 18% cyclic stretch, demethylation and inflammatory factors significantly regulate MYLK promoter activities. Two SNPs (rs2700408 and rs17114297) associated with LTL in AA and EA significantly influence transcription factors binding to the gene and regulate the promoter activity for gene transcription. SNP (rs57186134) significantly associated with asthma susceptibility. In luciferase assay, rs57186134 significantly increased MYLK promoter activity in endothelial cells. The binding of MYLK promoter to the growth factor independent 1 transcription repressor (GFI1) was significantly interrupted by rs57186134.

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Conclusion: These findings suggest that the MYLK gene is transcriptionally regulated by mechanical stress and inflammatory factors, and modulated by SNPs associated with lung inflammatory diseases. These functional insights further strengthen the concept that MYLK contributes to inflammatory disease susceptibility and represents a molecular target in complex lung disorders. (NIHBI HL058064 and HL091889)

ABSTRACT FINAL ID: 25

PARTICULATE MATTER EXACERBATES LUNG INFLAMMATION IN MICE WITH CARDIOMYOPATHY, A GENOMIC STUDY

T. Wang, T. Zhou, L. Moreno-Vinasco, V. Natarajan, J.G. Garcia. Department of Medicine, University of Illinois at Chicago, Chicago, IL.

Abstract Body:
Epidemiologic studies consistently demonstrate significant associations between the levels of ambient particulate matter (PM) air pollutants and cardiovascular mortality and morbidity, especially among the patients with congestive heart failure (CHF). The mechanism that mediates PM-exacerbated CHF susceptibility has not been identified. In this particular study, we utilize a murine model of CHF (CREB transgenic model) to elucidate the effects of PM exposure on the lungs, the first organ making direct contact with PM, with approaches focusing on both pathophysiology and toxicogenomics. The lung parameters are analyzed post PM exposure (36 hrs, 20 mg/kg, intratracheal instillation) BAL protein content, cellularity of infiltrated white blood cells, as well as inflammatory cytokine levels are quantified. Lung gene expression is also characterized on an Affymetrix microarray platform and validated by qPCR. Consistent with our previous findings, we observe significant increases in pulmonary inflammation in wild type CD-1 mice with PM exposure inducing significant elevations in BAL protein levels, white blood cell counts, and inflammatory cytokines IL-6 and TNFα. PM mediates significant more severe pulmonary inflammation in CREB mice with CHF compared to CD-1 mice in all inflammatory parameters. Furthermore, PM consistently induces exacerbated gene expression patterns in CREB mice compared to CD-1 mice. PM dysregulates 563 lung genes in CREB mice, while only 2 lung genes in CD-1 mice with the same filtration criteria (q-value <0.01 and fold change >1.5). In the lungs from CREB mice, PM significantly dysregulates signaling pathways including tight junction, focal adhesion, ubiquitin mediated proteolysis, leukocyte transendothelial migration. This study is consistent with emerging epidemiologic evidence and indicates that PM exposure evokes proinflammatory signatures in the population. Furthermore, these results demonstrate that PM exaggerates lung inflammation and vascular hyperpermeability in mice with pre-existing CHF; strongly suggesting that pathobiology inherent to the CHF phenotype could be exaggerated by heightened lung inflammation and vascular hyperpermeability induced by PM exposure. These studies were supported by EPA PM Center Grant # RD83241701 (JNG), NIH HL058064 (JNG), and Parker B. Francis Family Foundation (TW).

ABSTRACT FINAL ID: 29

CYTOSKELETAL AND JUNCTIONAL COMPLEX REARRANGEMENT MEDIATES PULMONARY ENDOTHELIAL BARRIER ENHANCEMENT BY FTY720 S-PHOSPHONATE


Abstract Body:
Rationale: Modulation of pulmonary vascular barrier function is an important clinical goal given the devastating effects of vascular leak in acute lung injury (ALI). We previously demonstrated that FTY720 S-phosphonate (Tyspineote/Tys), an analog of sphingosine 1-phosphate (SIP) and FTY720, has more potent pulmonary barrier protective effects than these agents in vitro and in the LPS and bleomycin models of mouse ALI. Moreover, Tys preserves expression of the barrier promoting SIP1 receptor (SIPR1), whereas SIP and FTY720 induce its ubiquitination and degradation. In this report, we further characterize the novel barrier promoting effects of Tys on intracellular signaling and junctional assembly formation in cultured human pulmonary endothelial cells (EC).

Methods/Results: Mechanistic experiments were performed in vitro using cultured human pulmonary EC. Reduced SIPR1 expression via siRNA significantly attenuated transendothelial electrical resistance (TER) elevation by Tys. Inhibition of cytoskeleton rearrangement by depolymerizing actin with cytochalasin blocked Tys-induced TER elevation. Tys significantly increased Rac1 activity, while inhibition of Rac1 activity by pharmacological inhibitor significantly attenuated Tys-induced TER elevation. Tys significantly increased phosphorylation and cell peripheral redistribution of the actin-binding protein, cortactin, while cortactin siRNA partially attenuated Tys-induced TER elevation. Furthermore, Tys significantly increased peripheral redistribution of adherens junction proteins-VE-cadherin and beta-catenin, and tight junction protein-ZO-1, ZO-1, ZO-2 or claudin 5 siRNA partially inhibited Tys-induced TER elevation, suggesting that tight junctions are involved in this process. Tys-induced barrier enhancement was significantly inhibited by VE-cadherin inhibitory antibody, suggesting that adherens junction complexes play a crucial role in this process.

Conclusion: Cytoskeletal and junctional complex rearrangement play critical roles in Tys-mediated barrier protection in pulmonary EC. These results provide mechanistic insights into the effects of this potential ALI therapy.
ABSTRACT FINAL ID: 47

MECHANISMS OF PROTEIN KINASE C DELTA-MEDIATED REGULATION OF THROMBIN-INDUCED HUMAN LUNG ENDOTHELIAL CELL BARRIER DISRUPTION


Abstract Body:
PKC plays a significant role in thrombin-induced loss of endothelial cell (EC) barrier integrity; however, the existence of more than 10 isozymes of PKC and tissue-specific isoform expression has limited our understanding of this important second messenger in vascular homeostasis. In this study, we show that PKC-delta isoform promotes thrombin-induced loss of human pulmonary artery EC (HPAEC) barrier integrity, findings substantiated by PKC-delta inhibitory studies (rotteni, dominant negative PKC-delta construct). In addition, we identified PKC-delta as a signaling mediator upstream of both thrombin-induced MLC phosphorylation and Rho GTPase activation affecting stress fiber formation, cell contraction and loss of EC barrier integrity. Our inhibitor-based studies indicate that thrombin-induced PKC-delta activation exerts a positive inotropic effect on HPAEC and contributes to Rac1 GTPase inhibition. Moreover, PKD (or PKC-mu) and CPI-17, two known PKC-delta targets, were found to be activated by PKC-delta in EC and served as modulators of cytoskeleton rearrangement. These studies clarify the role of PKC-delta in EC cytoskeleton regulation, and highlight PKC-delta as a therapeutic target in inflammatory lung disorders, characterized by the loss of barrier integrity, such as acute lung injury and sepsis.

ABSTRACT FINAL ID: 79

REDOX REGULATION OF STAT6 IS LINKED TO DEVELOPMENT OF PULMONARY FIBROSIS IN MICE

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Abstract Body:
M2-phenotype macrophages have an integral role in fibrosis development. We have shown that Cu,Zn-SOD mediates the decrease activation of STAT6 by the Cu,Zn-SOD enzyme. However, we have not been able to determine the role of Cu,Zn-SOD specific isoform expression has limited our understanding of this important second messenger in vascular homeostasis. In this study, we show that Cu,Zn-SOD induces H2O2 and that the Cu,Zn-SOD enzyme mediates the decrease activation of STAT6. We demonstrate that Cu,Zn-SOD mediates the decrease activation of STAT6. We also show that Cu,Zn-SOD mediates the decrease activation of STAT6 by Cu,Zn-SOD specific isoform expression has limited our understanding of this important second messenger in vascular homeostasis. In this study, we show that Cu,Zn-SOD induces H2O2 and that the Cu,Zn-SOD enzyme mediates the decrease activation of STAT6. We demonstrate that Cu,Zn-SOD mediates the decrease activation of STAT6.

ABSTRACT FINAL ID: 85

IL-13 MEDIATED TGF-β SECRETION IS DEPENDENT ON MITOCHONDRIAL ROS

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Abstract Body:
Background: A key component of asthma involves sub-epithelial fibrosis in response to airway injury and inflammation. Previous studies have shown that IL-13 mediated TGF-β secretion is dependent on mitochondrial ROS. Inhibition of mitochondrial ROS may be a novel target in future asthma therapies.

Methods: Animal Studies: Mice were treated with or without mitoTEMPO, an intracellular antioxidant. MitoTEMPO was administered intraperitoneally at 5 mg/kg/day through osmotic mini-pump and then subjected to an 8 day ovalbumin (OVA) sensitization protocol. Airway hyper-reactivity in response to methacholine was assessed using the Scribella Flexivent.

Results: A: Mitochondrial specific antioxidant therapy resulted in significant decreases in airway hyper-reactivity and sub-epithelial fibrosis measured by hydroxyproline. Cell Culture: Human bronchial epithelial cells (HBEC) were used to assess in-vitro response of epithelial cells to IL-13. Cells were pre-treated with mitoTEMPO 10 μM to determine the effect of mitochondrial ROS on IL-13 function. Mitochondrial superoxide was measured using mitoSOX Red (Invitrogen) and lucigenin chemiluminescence. Lucigenin was used to measure superoxide in isolated mitochondria. TGF-β was measured in media using a commercially available DuoSet ELISA development kit (R&D).

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Conclusions: Our data suggest that IL-13 mediated airway remodeling is dependent on mitochondrial ROS. Inhibition of mitochondrial ROS may be a novel target in future asthma therapies.
ABSTRACT FINAL ID: 87

ROLE OF MIF IN AGE-RELATED SUSCEPTIBILITY TO RADIATION LUNG INJURY VIA NF22 AND ANTI-OXIDANT REGULATION


Abstract Body:
Microvascular injury and increased vascular leak are prominent features of radiation-induced lung injury (RIILI) and often lead to cancer-associated thoracic irradiation. Our previous studies demonstrated that polymorphisms in the gene (MIF) encoding macrophage migratory inhibition factor (MIF), a multi-functional pleiotropic cytokine, confer susceptibility to acute inflammatory lung injury and increased vascular permeability, particularly in senescence. In this study, we explored total antioxidant levels (WT) and genetically engineered MIF−/− KO mice to 2 Gy single fraction thoracic radiation to investigate the age-related role of MIF in murine RIILI (ages 8 wks, 8 mos, 16 mos). Relative to 8wks, old mice decreased MIF expression was observed in bronchovascular lavage (BAL) fluid and lung tissues of 8-16 mos wildtype mice. We explored total antioxidant levels in BAL fluid from radiated mice and noted that 8-16 mos MIF−/− mice exhibit significantly decreased total antioxidant levels with progressive age-related decreases in nuclear expression of Nrf2, a transcription factor involved in antioxidant gene upregulation in response to reactive oxygen species. This was accompanied by decreases in both protein levels (NQO1, GCLC, heme oxygenase-1) and mRNA levels (Gpx1, Pdx1, Txn1) of Nrf2-influenced antioxidant gene targets. Additionally, MIF-silenced (siRNA) human pulmonary arterial endothelial cells failed to express Nrf2 following oxidative (H2O2) challenge, while Nrf2 was salvage by exogenously added recombinant MIF. However, treating with GSH but not NAC (an Nrf2 substrate), protected aged MIF−/− mice from RIILI. These findings implicate an important role for MIF in radiation-induced changes in lung cell antioxidant levels via Nrf2 and suggest that MIF may contribute to age-related susceptibility to the untoward effects of thoracic radiation.

ABSTRACT FINAL ID: 89

CD44 IS A NOVEL REGULATOR OF OXIDIZED PHOSPHOLIPIDS-MEDIATED RAC1 ACTIVATION IN CAVEOLIN-ENRICHED MICRODOMAINS AND PULMONARY ENDOTHELIAL BARRIER ENHANCEMENT

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Abstract Body:
Endothelial cell (EC) barrier dysfunction results in increased vascular permeability leading to increased leukocyte extravasation and mass transport across the vessel wall, crucial mechanisms in the pathogenesis of acute lung injury. We have previously demonstrated that oxidized 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphocholine (OxPAPC) significantly enhances vascular endothelial barrier properties in vitro and in vivo and attenuates endothelial hyper-permeability induced by inflammatory and edemagenic agents via Rac GTPase dependent mechanisms. These findings suggested potential important therapeutic value of barrier-protective oxidized phospholipids. In this study, we examined involvement of the EC barrier regulatory vascular endothelial growth factor (VEGF), cytoskeleton reorganization and EC barrier enhancement. Further, silencing (shRNA) expression blocked OxPAPC-mediated recruitment of cytoskeleton-2/ARNO recruitment to CEM, Arf6-dependent Rac1 activation and EC barrier enhancement. To confirm our in vitro results in an in vivo murine model of acute lung injury with pulmonary vascular hyper-permeability, we observed that selective silencing of CD44 expression in the lung vasculature blocked OxPAPC-mediated protection from lipopolysaccharide (LPS)-induced lung injury. Taken together, these results suggest CD44-dependent recruitment of paxillin and consequent cytohesin-2/ARNO activation of Arf6 within CEM is important for OxPAPC-mediated Rac1 activation and EC barrier protection.

RHEUMATOLOGY/IMMUNOLOGY/ALLERGY

ABSTRACT FINAL ID: 39

WHOLE BODY MAGNETIC RESONANCE IMAGING IN EVALUATION OF ENTHESIS IN SPONDYLOARTHROPATHY


Abstract Body:
Background: Enthesitis is a characteristic feature of spondyloarthritides, a spectrum of diseases. Positive tenderness at enthesial points constitutes clinical enthesitis. However, this may not always correlate with actual inflammation at entheses. Further, metatarsalgia or limited number of enthesial tenderness could be noted in healthy children. This study was undertaken to assess the agreement between enthesitis by physical examination and by whole body MRI (WB MRI).

Methods: Patients enrolled in “Studies of The Natural History and Pathogenesis of Spondyloarthritides” protocol who had undergone WB MRI were included in the study. Patients satisfied at least one of the following criteria: ERA, psoriatic arthritis (ILAR criteria); undifferentiated spondyloarthritides (Amor or ESSG criteria); axial spondyloarthritides (ASAS criteria); juvenile ankylosing spondylitis or adult ankylosing spondylitis (Modified New York criteria). Patients underwent detailed clinical evaluation including assessment of active joint count and manual palpation of 34 enthesial points. Inflammatorv markers, ANA and HLA-B27 were tested. Patients underwent WB MRI without contrast. Four healthy volunteers also underwent detailed clinical assessment and WB MRI. Median, IQR calculations were performed for descriptive statistical analysis. Kappa statistics was performed to assess agreement between clinical enthesial exam and WB MRI.

Results: Thirteen patients who had WB MRI; 66% of the patients were less than 16 years old at the time of symptom onset. The median disease duration was 36 months (IQR 15.7-108). Sixty nine percent of patients were males and 100% were Caucasian. Inflammatory back pain, as defined by ESSG criteria was present in 92% of patients; Schober was 5 (4.15-6.25). Sacroilitis by radiography was present in 3 patients. Median active joint count was 1 (IQR 0-3). Percentage of patients with arthritis and enthesitis was 61% and 69% respectively. Median ESR was 8 (IQR 5-21.5) and CRP was 0.52 (IQR 0.16-26.72). By exam, the most common positive enthesial sites were meral epicondyle, L5 spinous process, ASIS, plantar fascia insertion to MTP and 1st costosternal junction. A total of 108 enthesial sites were tested positive among patients. None of the patients had positive exam over the Achilles insertion. WB MRI detected synovial effusion in 6 patients; sacroilitis was detected in 4 patients. Greater trochanter of femur and iliac crest were the most common sites of enhancement on MRI. There was poor agreement between WB MRI and clinical exam for enthesitis when evaluated for all enthesial sites (kappa=.0). Assessment of kappa agreement (k) at individual enthesial sites was as follows: k= -0.13 at left greater trochanter; k= -0.226 at right greater trochanter, and k=0.235 at left iliac crest. Only one enthesial site was positive only on WB MRI and clinical exam. Among controls, total of 4 enthesial sites were tested positive by clinical exam and none of the controls had positive enthesitis by WB MRI.

Conclusion: This study informs us on the poor agreement between enthesitis by clinical examination and WB MRI. Clinical examination may overestimate the actual number of enthesial sites.

ABSTRACT FINAL ID: 91

GENETIC VARIATION NEAR IRF8 IS ASSOCIATED WITH SEROLOGY AND CYTOKINE PROFILES IN SYSTEMIC LUPUS ERYTHEMATOSUS AND MULTIPLE SCLEROSIS

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Adult-onset Still’s disease (ASD) is an inflammatory disorder typically characterized by high daily fevers, arthritis, and an evanescent rash. It is a rare condition, occurring in less than 1 per 100,000 people with a bimodal age distribution peaking between ages 15-25 and ages 36-46. Although an infectious origin is suspected, the etiology remains unproven. There is no definitive test or laboratory value to diagnose ASD. Several sets of criteria have been established to aid in the diagnosis, the most sensitive of which is the Yamaguchi criteria. The four major criteria include persistent high fever, leukocytosis, arthritis or arthralgia, and a skin rash that is usually present during the febrile episodes. The minor criteria include a sore throat, organomegaly, elevated liver function tests, lymphadenopathy, and normal anti-nuclear antibody and rheumatoid factor. In order to make the diagnosis, five of these features must be present, including two of the major criteria. Several treatment options are available, including nonsteroidal anti-inflammatory drugs, glucocorticoids, disease-modifying antirheumatic drugs, and biologic immunomodulatory agents. The disease can follow a variable time course, and the prognosis is generally favorable with only a small subset of patients suffering long-term complications.

In conclusion, adult-onset Still’s disease is an uncommon condition that in rare cases can present with myopericarditis.