significantly with DBS ON versus OFF (p=0.015). This decline was not significantly different from that measured in the lesioned hemispheres (p=0.7). **Conclusions:** Our findings indicate that STN DBS showed metabolic changes that were similar to those encountered with subthalamotomy, although post-operative decrements in the lentiform nuclei were greater with lesioning. Both STN procedures gave rise to significant operative reductions in PDRP network activity. Overall, our results suggest that STN DBS and subthalamotomy achieve therapeutic benefit through the modulation of the same spatially distributed metabolic pathways.

36

HEAT SHOCK PROTEIN 70 EXPRESSION IS REDUCED IN RAT MYOCARDIUM FOLLOWING TRANSMYOCARDIAL LASER REVASCULARIZATION. H. Ma, R. Huang, O. Abela, A. Maheshwari, G.S. Abela, Department of Medicine, Michigan State University, East Lansing, MI

Background: Transmyocardial laser revascularization (TMLR) has been shown to relieve symptomatic ischemia but laser tissue effects have potential complications. In order to define the mechanism of laser action, heat shock protein (hsp) expression was evaluated in rat hearts after TMLR. **Methods:** Under general anesthesia, hearts were removed from 10 rats and immediately placed in oxygenated physiologic buffered solution (PBS) at 0°C. After the various treatments, hearts were homogenized and hsp70 was measured with Western Blotting. Group 1 (n=3) hearts were immediately homogenized; Group 2 (n=3) hearts were perfused with the PBS in a Langendorff setup for 6 h; Group 3 (n=3) hearts were lased (50 channels) using a Ho:YAG laser via a 0.6 mm core fiber at 3 Hz and 280 mJ/pulse and perfused up to 6 h. Group 4 (n=1) heart was heated at 42°C for 15 min then recovered at 23°C for 6h prior to hsp measurement. **Results:** There was a significantly lower hsp70 expression in Group 3 (TMLR) and higher in Group 4 than that the control Groups (1, 2). **Conclusion and Discussion:** In isolated rat hearts under stress, lasing lowered the expression of hsp70. This could be related to laser inhibition of hsp70 expression or enhancement of its degradation. TMLR may protect myocardial cells from stress related expression of hsp.

	Group 1*	Group 2*	Group 3**	Group 4
Relative OD_{600nm}	1	0.87±0.10	0.19±0.03	3.47

* to *: p=ns; * to **: p<0.003.

37

INSULIN'S ABILITY TO SUPPRESS ADIPONECTIN *IN VIVO* IN HUMANS IS INDEPENDENT OF ENDOTHELIN ACTIVITY. L. Brame, R.V. Considine, M. Yamauchi, A.D. Baron, K.J. Mather, Department of Medicine, Indiana University, Indianapolis, IN

The circulating levels of adiponectin, a multimeric polypeptide produced by adipocytes, are inversely proportional to insulin sensitivity and are suppressed by insulin infusions *in vivo*. Endothelin is a peptide produced by the vascular endothelium which appears to have effects on insulin sensitivity via direct cellular effects and via vascular effects. Obesity is characterized by insulin resistance, reduced adiponectin levels, and increased endogenous endothelin activity. We therefore hypothesized that endothelin activity would modulate insulin's ability to suppress adiponectin *in vivo* in obese humans We studied 17 lean and 20 obese (BMI 21.8±2.2 and 34.5±5.6 kg/m^2 respectively) subjects. Hyperinsulinemic euglycemic clamp studies were performed using insulin infusion rates of 10, 30 or 300 mU/m2/min either alone or with concurrent infusion of BQ123, a highly specific antagonist of type A endothelin receptors. Circulating adiponectin levels were assessed at baseline and following achievement of steady state glucose infusion under hyperinsulinemia. Paired data from insulin infusion studies with and without BQ123 were available for 13 lean and 14 obese subjects. The remainder participated in only one of these studies. Adiponectin levels were lower in obese than lean subjects (6.6±3.6 vs. 8.3±2.8 μg/mL, p=0.04). In all subjects, insulin infusions significantly suppressed adiponectin by ~7% (p<0.0001). Suppression in obese and lean subjects was not significantly different (p=NS). By repeated measures ANOVA (using full paired data only), there was no evident effect of BQ123 to modulate insulin suppression of adiponectin (p=0.16), with no difference between lean and obese subjects (p=NS). Surprisingly, there was no evident relationship between steady state insulin concentrations and adiponectin suppression (r=0.14, p=NS), and again no effect of BQ123 to modify this relationship was seen. In conclusion, despite baseline differences in adiponectin status we observed equal suppression of adiponectin with insulin infusions in lean and obese subjects. Endothelin antagonism did not modulate this effect, suggesting that endothelin's effects on insulin sensitivity are independent of adiponectin and arguing against an effect of endothelin to regulate adiponectin biology. The lack of an evident acute dose-dependency of insulin's effect on adiponectin may suggest that hyperinsulinemia suppresses adiponectin levels indirectly.

38

SPHINGOSINE 1-PHOSPHATE REDUCES REGIONAL ALVEOLAR EDEMA FORMATION IN A CANINE MODEL OF LPS-MEDIATED ACUTE LUNG INJURY. B.J. McVerry, B. A. Simon, J.G.N. Garcia, Johns Hopkins University School of Medicine, Baltimore, MD

Purpose: High permeability pulmonary edema is a key feature of acute lung injury (ALI), and a significant cause of morbidity and mortality in critically ill patients. We previously demonstrated that sphingosine 1-phosphate (S1P), a biologically active phospholipid, enhances endothelial layer barrier function in vitro and attenuates pulmonary vascular permeability in LPS-injured mice. We hypothesized that S1P would attenuate regional alveolar edema formation in a canine model of LPS-induced ALI. **Methods:** Eight male beagles (12-18 kg) were anesthetized and mechanically ventilated (MV). LPS (2 mg/kg in 0.9 NS) was segmentally instilled intrabronchially via fiberoptic bronchoscope in 4 control dogs and in 4 dogs treated concomitantly with intravenous S1P (85 μg/kg). Supportive care including MV (Vt=17cc/kg, PEEP=5 cm H2O, FiO2=30%), fluid resuscitation, and hemodynamic monitoring was provided for 8 hours. ALI was quantified by shunt formation (Qs/Qt) and bronchoalveolar lavage (BAL) protein concentration. CT quantification of regional lung water/tissue content was performed after 6 hours. **Results:** All dogs remained hemodynamically stable. Qs/Qt increased rapidly following LPS instillation and continued to rise over time in control dogs. S1P attenuated increases in Qs/Qt induced by LPS (p<0.05 after 5h). BAL protein concentration was lower in S1P-treated dogs compared to controls at all time points. CT-derived lung water content was reduced in S1P-treated dogs compared to LPS controls (35% vs. 57% respectively) and was similar to normal canine lung historical controls (33%). **Conclusions:** Intravenous S1P attenuates regional edema formation associated with LPS-induced ALI. These results suggest that vascular leak reduction by S1P may shorten the duration of MV and therefore morbidity associated with ALI in critically ill patients.

39

D-DIMER LEVELS AS A CONSEQUENCE OF CORONARY ARTERIOGRAPHY. A. Mehrotra, A. Maheshwari, H. Hassouna, Michigan State University, East Lansing, MI

Background: In coronary arteriography, catheters are advanced from the femoral artery in a retrograde manner, and vessels are visualized by radio opaque contrast. Intravascularly, guide wires catheters and contrast should create a thrombogenic environment; however there are no reports documenting clotting and fibrinolysis potential during the procedure. **Methods:** D-dimer, a molecular marker of both thrombin activity on factor XIII and plasmin mediated fibrin degradation, was measured in 6 females and 14 males ages 42-65 years, selected at random from a list of patients scheduled for first time coronary arteriography, with each acting as their own control for baseline D-dimer values. After approval was obtained from institutional ethical review boards and informed consent was taken from all subjects, 5ml blood, drawn in 3.2% sodium citrate 1 hour pre- and 6 hours post-procedure, was immediately spun at 10,000 rpm for 20 minutes at 4°C and platelet poor plasma stored at –80°C until assayed. Samples were collected over 35 days and assays for all the subjects were performed on the same day and batched to minimize intra assay variability. ELISA was performed by sandwich technique using a polyclonal capture antibody for fixation of fibrinogen and fibrin degradation products and an activation dependent horseradish peroxidase bound monoclonal antibody against human D-dimer for quantification. Color formed by horseradish peroxidase reaction with O-phenylene diamine substrate was read at 492nm on Spectra Max Plus plate reader. Cut off point for the assay was 250 mg/L. Standard curves were derived by linear regression analysis for the changes in absorbance plotted against 2g/L, 1g/L, 500mg/L, and 250mg/L antigen-antibody-enzyme complex. Quantities of D-dimer units were estimated from changes in absorbance using corresponding points on standard curve. **Results:** D-dimer pre-catheterization levels among all 20 patients show variability ranging from baseline (250 mg/ L) up to 390 mg/L. ANOVA is run for D-dimer using repeated-measures analysis of variance for each patient with (pre-and post catheterization levels) as the within subjects factor. Specific effects of age, race, weight and severity of cardiovascular disease are being assessed. Data will be presented to show statistically significant increase above baseline in D-dimer for all patients. **Interpretation:** Increase in products of fibrin degradation after angiography is suggestive of release of tissue factor and thrombin systemically with clot formation and subsequent fibrinolysis. Coronary angiography creates a thrombogenic environment and that significant thrombosis is prevented by constant clot dissolution. *Research supported by AMA Seed Research Grant to Avanti Mehrotra.*

40

NF-KAPPA B AND P38 CROSS-TALK IS CRITICAL FOR OSTEOCLAST DIFFERENTIATION. C. Menaa, M. Corr, C.J. Froelich, S.M. Sprague, Evanston Northwestern Healthcare, Northwestern University, Evanston, IL

RANKL is the key cytokine that regulates osteoclast (OC) differentiation. RANKL binds to its receptor, RANK, which then mediates the activation of NF-κB and various MAP kinases, especially p38 MAPK and JNK. The role of NF-κB and JNK in OC biology have been highlighted by *in vivo* studies using knock-out mice. In *in vitro* studies utilizing specific inhibitors a regulatory role of the p38 MAPK has been demonstrated. *In vitro* experiments have also demonstrated that NF-κB and p38 are regulated by both activators and inhibitors of OC formation suggesting possible cross-talk between these two signal pathways. However, at what step of OC formation and whether the activation of these signaling pathways is synchronized are not known. In order to differentiate the role of the p38 MAPK, we first developed stable cell lines of an OC precursor (RAW 264.7) which over-express dominant negatives (DN) of the upstream kinases regulating p38 MAPK, KK6 and KK3. While the *in vitro* assay demonstrated that the DNs block the effect of immuno-precipitated KK6 or KK3 on p38 MAPK, OC formation was not affected. Moreover, double transfected cells which over-expressed both the DN of KK6 and KK3, were still capable of forming OC in response to RANKL. These findings suggest that an alternative pathway might account for the critical role of p38 MAPK during OC formation. However, the inhibition of NF-κB activity by over-expression of the super-repressor IκB mutant, totally blocked OC formation in response to RANKL. This effect was due to a shift in RAW cell differentiation pathway, rather than to an absolute reduction in numbers of OC precursors, which could be related to either an inhibition in their proliferation or an increase cell death. More importantly, this effect was not due to an impaired RANK receptor but rather to a specific effect of NF-κB, because the IκB super-repressor does not block RANKL stimulating NF-ATc1. Further analysis demonstrated that the p38 MAPK activation was impaired suggesting that the inhibitory effect of NF-κB might be due to the inhibition of p38 MAPK. In summary, these data demonstrated that NF-κB is critical for OC differentiation even during the over-expression of NF-ATc1 and that p38 MAPK may mediate this effect. In conclusion, these results collectively suggest that the cross-talk between NF-κB and p38 MAPK is essential for osteoclastogenesis. Thus, our data provide new insight on how multiple signaling pathways of RANKL are synchronized.

41

CHEMOKINE INDUCTION AND CELL SIGNALING BY ADENOVIRUS IN A HUMAN LUNG SLICE MODEL. J.P. Metcalf, J.L. Booth, V.R. Oblander, B.E. Gordon, K.M. Coggeshall, Pulmonary and Critical Care Division, Department of Medicine, Univesity of Oklahoma Health Sciences Center, and the Program in Immunobiology and Cancer, Oklahoma Medical Research Foundation, Oklahoma City, OK

Adenovirus (Ad) causes respiratory illness in immunocompetent and immunocompromised adults and children. There are type-specific consequences of infection with Ad as subgroup B, particularly type 7 Ad is associated with severe lung infection and pneumonia while other subgroup viruses cause upper respiratory tract infection but not pneumonia. The initial response to Ad in animal models is neutrophilic inflammation of the distal airways with a neutrophilic alveolitis. Interleukin-8 (IL-8) is the major neutrophil chemotaxin in the lung and we have shown that Ad7 induces IL-8 in A549 alveolar epithelial cells and in a human lung slice model. The purpose of this study was to determine if the lung slice model could be used to evaluate the role of cell signaling in IL-8 induction by Ad. We focused on extracellular regulated kinase (Erk), an important mediator of IL-8 induction.