studied using global OGG1$^{-/-}$ mice and novel VSMC-specific transgenic mice lines expressing OGG1 or $^{K338/K341}$OGG1. BER activity was measured via incision cleavage of an 8-oxoG-containing oligonucleotide, 8-oxoG levels by ELISA, and protein binding/interaction by immunoprecipitation and western blotting. OGG1, acetyl OGG1, p300, and 8-oxoG expression were assayed in mouse arteries and human plaques by immunohistochemistry.

Results Nuclear BER activity was defective in atherosclerotic plaque VSMCs, which correlated with a concomitant decrease in acetylation of OGG1 and OGG1 protein expression. *In vitro* knockout of OGG1 by CRISPR/CAS9 indicated that OGG1 is the major BER DNA glycosylase in VSMCs. Consistently, OGG1 expression increased BER activity and reduced 8-oxoG levels *in vitro* and *in vivo*, features not seen with the $^{K338/K341}$OGG1 acetylation mutant, indicating that acetylation regulates OGG1 activity in human VSMCs.

OGG1 knockout cells displayed increased sensitivity to DNA breaks induced by oxidant stress and increased apoptosis compared to control cells, measured by comet assay and Annexin-V/propidium iodide FACS staining respectively. This effect was blunted by overexpression of OGG1 but not $^{K338/K341}$OGG1, suggesting a protective effect of OGG1 against DNA damage, and that the acetylation status of OGG1 is crucial for its function. Immunoprecipitation studies showed that p300 binds to OGG1, and this interaction was reduced under oxidative stress. Furthermore, levels of p300 were decreased in human plaque VSMCs and in response to oxidative stress, suggesting that ROS-induced regulation of OGG1 acetylation could be due to ROS-induced decreased in p300 expression and regulation of its interaction with OGG1.

Conclusions In conclusion, nuclear BER activity is reduced in human atherosclerotic plaque VSMCs, associated with reduced OGG1 acetylation and activity, potentially mediated by reduced p300. Mice with decreased and increased OGG1 will be used to study whether oxidative damage promotes plaque progression in atherosclerosis.

166 CHANGES IN E-C COUPLING PROTEINS AND TRANSVERSE AND AXIAL TUBULAR STRUCTURES IN GUINEA PIG VENTRICULAR MUSCLE DURING PRE- AND POST-NATAL DEVELOPMENT TO ADULTHOOD

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Transverse tubules (TT), plasmalemma invaginations perpendicular to the long axis of the cardiomyocyte, facilitate rapid action potential transmission to the cell interior and efficient cardiac excitation-contraction coupling (ECC). Tubule alteration and changes in expression of proteins important for normal ECC have been noted in heart disease. The aim of this study is to explore the changes of selected ECC-related protein expressions, and transverse tubular structures, from pre- and post-natal cardiac development to adulthood in guinea pig ventricular muscle. Hearts were collected from guinea pigs at developmental stages: fetal (between gestation days (G) 55–68; term=G67–68); neonatal week one (NW1), two (NW2) and three (NW3); and adult. Hearts were flushed with a cold cardiologic solution and (i) left ventricles (LV) frozen for subsequent protein analysis by western blotting or (ii) retrogradely perfused with fixative and LV processed for ultrastructural examination by serial block face-scanning electron microscopy. Values are mean± SEM, compared by one-way ANOVA and Bonferroni posthoc test ($p<0.05$). Expression of Î² adrenoceptor, TT marker, increased in adults (0.87 ±0.14 fold relative to positive control) and neonates NW2 (0.90±0.04 fold) compared to fetal G55/57 (0.44±0.04 fold) or neonates NW1 (0.5±0.04 fold). Junctophilin2, a determinant of TT integrity, was expressed in G55/57 and, surprisingly, was invariant among the biological groupings. Analysis of digitally reconstructed (Amira 6.0) serial EM images revealed developmental changes in cardiomyocyte structure. Sarcomere length narrowed from G55/57 (2.28±0.01 μm) to NW1 (1.90±0.01μm) and adult (1.92±0.01 μm); assessing 250 sarcomeres. Total tubular volume increased from 0.68 ±0.08% at G64/68 to 2.54±0.48% in the adult. Fetal cardiac TTs appear as early as the mid-third trimester of guinea pig pregnancy. Changes in TT/TAT abundance through pre- and post-natal development to adulthood mirror the changes in expression of some (Î² adrenoceptor) but not other (junctophilin2) proteins likely to be important for maturation of ECC. TT formation may start prenatally but it is less organised.

167 LEPTIN INDUCES SCA-1+ PROGENITOR CELL MIGRATION ENHANCING NEOINTIMAL FORMATION

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Background Leptin is a hormone that is predominantly secreted by white adipose tissue and thus the majority of obese individuals display high concentrations of plasma leptin. Initially believed to be solely a metabolic factor, leptin also plays a role in inflammation, vascular disease, to which Sca-1 + vascular progenitor cells within the vessel wall may contribute.

Hypothesis Leptin can influence neointimal formation by promoting the migration of Sca-1+ progenitor cells.

Methods and results Sca-1+ progenitor cells were cultivated from the vessel wall of apoE-/- mice and purified via microbeads. The cell migration assessments included transwell and wound healing assays *in vitro*. The migration of Sca-1+ progenitor cells was markedly induced by leptin in a dose-dependent manner (2.420±0.222 and 1.318±0.036). This migration induced by leptin was significantly inhibited by a leptin triple mutant antagonist CYT-566, ERK inhibitor or a STAT3 inhibitor. Western blot analysis revealed that leptin induced phosphorylation of STAT3 and ERK1/2, respectively, implicating the impact of these signal pathways. When applied the Sca-1+ progenitor cells from leptin receptor deficient mice for the experiments above, both the migratory ability and protein activation were markedly abolished. When endovascular injury was induced in wild-type mice by passing a 0.014 guidewire three times through the femoral artery, neointimal lesions were observed in a time-dependent manner. Immunostaining for leptin displayed an increased expression of leptin in the injured vessels. Serum ELISA for leptin assay demonstrated a peak increase at 24 hours after vessel injury. When red fluorescent protein (RFP) labelled-Sca-1+

progenitor cells in Matrigel were applied to the adventitia of the injured femoral artery, REP+ cells were observed in the intimal 24 hours post-operation, which significantly enhanced neointimal lesions at 2 weeks. This increased neointimal lesion was rescued by pre-treatment of Sca-1+ cells with leptin antagonist CYT-566. In addition, when wild-type Sca-1+ progenitor cells were delivered to the adventitia of the injured artery in leptin receptor deficient mice, a significant increased neointimal formation was observed at 2 weeks, indicating crucial roles of leptin receptor and Sca-1+ progenitor cells in vascular remodelling.

Conclusions- Levels of leptin in both the vessel wall and the circulation are upregulated after vessel injury, leading to the migration of Sca-1+ progenitor cells that enhances neointimal formation.

168 CARDIOPROTECTIVE ROLE OF FINGOLIMOD IN ISCHEMIA-REPERFUSION INJURY BY ACTIVATION OF AKT/ERK PATHWAYS IN RAT MODEL

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Background and purpose Ischaemic Heart Diseases (IHD) are the most common cause of morbidity and mortality. Incidence and prevalence are continuously growing. There is an escalating risk for revascularisation or resuscitation in patients with IHD. Recently, it has been reported that a sphingosine 1-phosphate receptor agonist play an anti-apoptotic and anti-inflammatory role in the ischemia-reperfusion injury.

Objectives The aim of our study is to investigate the cardioprotective effects of sphingosine 1-phosphate receptor agonist fingolimod (FTY720) on global ischemia-reperfusion injury related to the cardiac surgery.

Methods In our experimental study, global ischemia-reperfusion was achieved by cardiopulmonary bypass by cardioplegic arrest on ventilated male Sprague-Dawley rats (300–350 g). The global ischaemic period lasted 10 min in the cardioplegic arrest while reperfusion times were maintained for 60 min and 24 hours. ECG monitoring was done using AD instrument and using Millar catheter, heart rate, systolic and diastolic pressures were recorded and mean arterial pressure was calculated. The statistical significance was considered at P 0.05.

Results The myocardial protection was observed in the group treated with Fingolimod as compared to control groups. Reduced frequency of apoptotic cells and inflammatory mediators were found in the treated group. The level of adenylates was preserved in the treated group as compared to controls (94%, 61% respectively)(p0.001). Reactive Oxygen Species (ROS) were attenuated in the fingolimod-treated group. Fingolimod treatment improved systolic and diastolic ventricular pressures and contractility strength (p0.005).

Conclusions The intravenous administration of fingolimod in global ischemia-reperfusion was cardioprotective. Fingolimod cardioprotection appears to be mediated through preservation of high energy phosphates, reduction in oxidative stress, inhibition of apoptosis and inflammation leading to improved cardiac functions.

169 DEVELOPING CAPTURE ASSAYS TO MEASURE CIRCULATING CRP/OXIDISED LOW DENSITY LIPOPROTEIN COMPLEXES IN PATIENTS UNDERGOING CARDIOPULMONARY BYPASS AND EXAMINING USE OF CRP/OXIDISED LOW DENSITY LIPOPROTEIN COMPLEXES AS A BIOMARKER OF ATHEROSCLEROSIS

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Background Atherosclerosis is a disease of global importance. It is a systemic chronic inflammatory disease with a complex aetiology. LDL is primarily oxidised in the subendothelial space, as plasma antioxidants protect circulating LDL from oxidation. However, small amounts of oxidised LDL (oxLDL) have been detected in plasma. Modified LDL is thought to circulate as part of an immune complex; these immune complexes may also include CRP, complement and IgM antibodies. This project aims to develop a capture ELISA to measure oxLDL/CRP complexes in circulation, to help better characterise circulating immune complexes and explore potential use of oxLDL/CRP complexes a biomarker of atherosclerosis.

Methods A CRP/oxLDL capture ELISA was developed using LO1, a novel mAb specific to oxLDL. Plasma samples were obtained from patients undergoing cardiopulmonary bypass at four different timepoints. Levels of oxLDL, CRP and oxLDL/CRP complexes were determined in patient plasma using capture assays developed during this study.

Results OxLDL levels show an uptrend from baseline to 60mins, followed by significant reduction at 300mins compared to baseline (0.506 ± 0.078 vs 0.456 ± 0.035; $p=0.0138$). CRP concentration initially declines from baseline up to 120mins (1.918 ± 0.915 vs 1.136 ± 0.599; $p=0.0009$), after which levels rise between 120 min and 300 min (1.136 ± 0.599 vs 1.782 ± 0.65; $p=0.0026$). There is no significant difference in levels of CRP/oxLDL complexes across all timepoints.

Conclusion It is possible to measure CRP/oxLDL complexes in plasma. Pro-inflammatory events such as cardiopulmonary bypass are associated with changes in levels of oxLDL and CRP, while oxLDL/CRP levels remain unchanged.

170 FETUIN-A INDUCES NOX1-ROS-DEPENDENT VASCULAR DYSFUNCTION PARTIALLY THROUGH TOLL LIKE RECEPTOR 4 ACTIVATION

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Fetuin-A (FetA) is a plasma glycoprotein important for the regulation of calcium and phosphate homeostasis. It is also an agonist to toll-like receptor 4 (TLR4) and is related to insulin resistance and inflammation. FetA has also been associated with endothelial dysfunction, which is regulated by oxidative stress. Mechanisms whereby FetA influences vascular function are unknown. We hypothesised that FetA through TLR4 and ROS production induces vascular dysfunction. Mesenteric