signalling could be potential therapies for the prevention of vascular calcification.

## 226 THE IMPACT OF NADPH OXIDASE 2 INHIBITION ON SKELETAL MUSCLE PATHOPHYSIOLOGY OF ATHEROSCLEROTIC MICE

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**Introduction** Apolipoprotein E-deficient mice ($ApoE^{-/-}$) develop severe hyperlipidaemia and atherosclerotic lesions throughout the aortic root, features that are aggravated by atherogenic diets. NADPH oxidase 2 (Nox2) is an important enzymatic source of reactive oxygen species that contributes to systemic atherosclerosis in $ApoE^{-/-}$ mice. The aim of this study was to investigate the role of Nox2 inhibition in skeletal muscle pathophysiology and cellular oxidative stress of $ApoE^{-/-}$ mice administered a Western-type of diet (WD).

**Methods** $ApoE^{-/-}$ mice were maintained on either a chow or a Western diet for 12 weeks and were treated with the Nox2dstat inhibitor or control peptide for the last 8 weeks of feeding. Skeletal muscles and the liver were dissected for molecular, biochemical and histological analysis.

**Results** Individual muscle fibres from $ApoE^{-/-}$ mice were significantly enlarged due to ectopic fat accumulation. There was an increase in hepatic inflammation and lipid deposition in response to WD administration. Importantly, there was perturbed gene expression for fatty acid metabolism and antioxidant genes, followed by evidence of oxidative stress, as shown by elevated lipid peroxidation and oxidative protein modifications such as carbonylation and tyrosine nitration in the skeletal muscle of WD-fed mice. Pharmacological inhibition of Nox2 decreased superoxide production and protein carbonylation, one of the most harmful protein modifications, in the muscle of $ApoE^{-/-}$ mice but had no effect on the liver.

**Conclusions** Our data indicate that ApoE deficiency induces oxidative damage in skeletal muscle and hepatic steatosis that are more profound under Western diet. Nox2 inhibition attenuates oxidative stress in skeletal muscle and holds promise for counteracting the impact of peripheral atherosclerosis in skeletal muscle. This study provides key evidence to better understand the pathophysiology of skeletal muscle in peripheral atherosclerosis and arterial disease; it also identifies alternative therapies to combat muscle oxidative stress.

## 227 INFARCT SIZE IN A RAT MODEL OF ACUTE MYOCARDIAL INFARCTION IS REDUCED BY INTERLEUKIN-6 TRANS-SIGNALLING BLOCKADE USING SGP130FC BUT NOT AN ANTI-IL-6R MONOCLONAL ANTIBODY

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**Introduction** Interleukin-6 (IL-6) is elevated during acute myocardial infarction (AMI) particularly after reperfusion with primary percutaneous coronary intervention (PPCI). Higher circulating levels of IL-6 and its soluble receptor (sIL-6R) are associated with adverse outcomes post AMI. Therefore while IL-6 is a potential therapeutic target in AMI, animal models employing monoclonal antibodies (MAb) against the IL-6R have failed to demonstrate benefit. We hypothesised that blockade of the pro-inflammatory aspects of IL-6 signalling (trans-signalling) with the sgp130Fc protein in an animal model of AMI would result in reduced infarct size (IS) whereas blockade with a MAb against IL-6R (which blocks both the pro and anti-inflammatory actions of IL-6) would not.

**Methods** AMI was induced in male Sprague-Dawley rats by occluding the left-anterior descending artery for 50 minutes prior to reperfusion (analogous to PPCI). The model was characterised by measuring the temporal profile of IL-6, sIL-6R and other inflammatory mediators (MCP-1, KC/GRO, IL-1β, TNFα) within the heart tissue and plasma by ELISA at 2, 4, 24, 72, 120 and 168 hours post AMI (n=3–4/group). In addition, infarct progression over time (measured histologically with TTC and Evans Blue dyes and with plasma myoglobin), and leukocyte infiltration (flow-cytometry of cells obtained from heart digests) were measured. In therapeutic experiments rats received either 4 μg/g of a MAb against IL-6R (clone 15A7), 0.5 μg/g of sgp130Fc or vehicle alone given intravenously 1 minute prior to reperfusion (n=7–8/group).

**Results** IS/Area at risk (AAR) increased from 31.81% at 4 hours to 46.1% at 24 hours (p=0.03), with no further change at 48 hours. Myoglobin peaked at 24 hours. IL-6 levels in the heart were biphasic; a robust early peak at 2–4 hours was followed by a trough at 24 hours, and a more sustained peak between days 3–5. Only the early peak was associated with significantly elevated circulating IL-6. The early peak was temporally associated with infarct progression and neutrophil influx, whereas the second was associated with classical mononcyte infiltration. Other inflammatory mediators followed a similar but less pronounced biphasic pattern. Cardiac and plasma sIL-6R peaked at 24 hours, coinciding with maximal cardiac neutrophil numbers. Based on these data the effect of IL-6 antagonism was assessed at 24 hours. IS/AAR after blockade with anti-IL-6R MAb was unchanged compared with control (46.8% vs 46.1%). However, blockade with sgp130Fc resulted in a substantial reduction in IS/ARR (26.32%, p 0.0004).

**Conclusions** IL-6 trans-signalling blockade with sgp130Fc but not blockade with an anti-IL-6R MAb reduces IS/AAR in an animal model of AMI with reperfusion. Ongoing experiments seek to understand the mechanisms underpinning this observation and to explore the effects on infarct healing and remodelling.

## 228 BRAF INHIBITORS, SB590885 AND DABRAFENIB, ENHANCE ERK1/2 SIGNALLING IN CARDIOMYOCYTES AND PROMOTE CARDIAC HYPERTROPHY

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**Purpose** ERK1/2 are phosphorylated and activated by MKK1/2 that are phosphorylated and activated by Raf kinases. ERK1/2 promote cardiomyocyte hypertrophy and is protective, whereas