Conclusion The data show that higher levels of IgM antibodies, including IgM anti-MDA, are associated with a decreased coronary necrotic core volume and lipid core burden, whereas total serum IgG and IgG anti-MDA LDL antibodies were not related to the measured plaque characteristics. The ability of IgM antibodies but not HDL- or LDL-cholesterol levels to indicate these important plaque characteristics is consistent with a proposed mechanistic role.

156 INDUCERS OF PULMONARY ARTERIAL HYPERTENSION UPREGULATE THE EXPRESSION OF PLASMA MEMBRANE CALCIUM ATPASE 1 IN PULMONARY ARTERY SMOOTH MUSCLE CELLS

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Pulmonary arterial hypertension (PAH) is a chronic and life-threatening disease with high morbidity and mortality in adult and paediatric patients. PAH is characterised by a progressive narrowing and occlusion of small pulmonary arteries leading to increased pulmonary resistance, right ventricular hypertrophy, and, finally, right ventricular failure.

A large body of data has shown that proliferation and migration of pulmonary arterial smooth muscle cells (PASMCs) represent key events in the vascular remodelling of pulmonary arteries that occurs during PAH. Levels of cytoplasmic calcium are an important determinant of PASMC proliferation and migration, and failure in maintaining appropriate levels of intracellular calcium are associated with PAH. The plasma membrane calcium ATPase (PMCA) proteins extrude calcium from the cytosol to the extracellular medium, and in doing so, play a critical role in the modulation of intracellular calcium levels. In this work, we have investigated whether inducers of PAH trigger any changes in the expression of PMCA proteins in PASMCs.

Analysis of RNA expression levels for *PMCA* genes has revealed that treatment of PASMCs with PDGF results in a significant increase in the level of the RNA encoding for the protein PMCA1. Interestingly, *PMCA1* RNA levels were also elevated in lungs of rats with monocrotaline-induced PAH. No changes were observed in the RNA levels for PMCA4, the other major PMCA isoform expressed in PASMCs. Although previous studies on the regulation of *PMCA1* gene expression have identified functional binding sites for the transcription factors NFAT in the *PMCA1* promoter region, we show here that PDGF-mediated upregulation of *PMCA1* transcriptional expression is independent of activation of the calcineurin/NFAT signalling pathway.

Our results suggest the involvement of PMCA1 in PASMC deregulation during PAH, although determination of the link between increased expression of *PMCA1* and PAH requires further investigation.

157 MYELOID EXPRESSION OF TRIB1 REGULATES THE POLARISATION STATE OF TISSUE RESIDENT MACROPHAGES THAT HAS CONSEQUENCES ON PLASMA LIPID AND METABOLIC HOMEOSTASIS

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Introduction Genome wide association studies have identified Tribbles-1 (*TRIB1*) to be significantly associated with all major plasma lipid traits and as a risk factor for ischaemic heart disease and myocardial infarction. Studies in mice using *Trib1* full body KO and liver-specific over-expression and KO models have shown that hepatic expression of TRIB1 reduces circulating lipids. Additionally, *Trib1* has been implicated as a regulator of alternatively activated macrophages. However the potential interplay between hepatocytes, macrophages and *Trib1* remain unexplored.

This study aimed to assess whether myeloid *Trib1* regulates tissue macrophage polarisation and investigate its consequences on plasma lipid homeostasis.

Methods We developed myeloid specific *Trib1* conditional knockout (*Trib1 fl/fl* x *Lyz2Cre*; $Trib1^{KO}$) and over-expressor mice (*ROSA26Trib1.Tg* x *Lyz2Cre*; $Trib1^{Tg}$), thereby deleting or over-expressing *Trib1* in myeloid cells. Plasma lipid levels were directly measured by ion mobility. Macrophage phenotype was characterised in the liver (Kupffer cells, KCs), adipose (ATMs) and BMDMs by qPCR and semi-quantitative immunofluorescence analysis. Western blotting was used to assess regulators of macrophage polarisation. Furthermore, microarray analysis of human monocyte derived macrophages (MDMs) was employed to identify potential *TRIB1*-regulated cytokines.

Results Loss of myeloid *Trib1* increased levels of plasma triglyceride, VLDL-C ($p<0.05$) and promoted pro-inflammatory polarisation in KCs ($p<0.01$), ATMs ($p<0.01$) and BMDMs ($p<0.05$), while $Trib1^{Tg}$ mice revealed opposing changes in all parameters assessed. Western blotting showed TRIB1 modulates protein levels of C/EBP-β^2 and $-\beta^2$ ($p<0.05$), both key regulators of macrophage polarisation, via the control of COP1 activity and miR-155 expression. Microarray analysis of MDMs indicated *TRIB1* may regulate production of a number of pro-inflammatory cytokines that are implicated in fatty liver disease and adipocyte lipolysis. Reduced expression of these was confirmed in in $Trib1^{Tg}$ BMDMs ($p<0.05$).

Conclusions Myeloid *Trib1* is a potent regulator of lipid homeostasis, the loss of which promotes inflammation in metabolic tissues. Our observations uncover a novel mechanism of KC-hepatocyte cross talk mediated through *Trib1*.

158 QUANTIFYING MANGANESE-CALCIUM INTERACTION FOR OPTIMAL CARDIAC MANGANESE ENHANCED MRI

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Introduction Information on myocardial viability is important for the management of ischaemic cardiomyopathy patient. Difficulties in assessing viability arise because necrotic tissue and viable myocardium overlap at the infarct border zone. Manganese-enhanced MRI (MEMRI) is sensitive to viability and the inotropic state of the heart[1]. Mn^{2+} enters viable cardiomyocytes via Ca^{2+} channels and enhances intracellular T1 relaxation. As intracellular Ca^{2+} is a central regulator of cardiac contractility high Mn^{2+} concentration can be cardiotoxic. Here, Mn^{2+} salt has been supplemented with Ca-gluconate to provide a Calcium supplement that should overcome inotropy while still providing enhancement of viable myocardium.

Methods/Materials Two manganese-based media were used: 50 mM $MnCl_2$ in saline and 50 mM $MnCl_2$ in a 1:3 ratio with 58 mM Ca-gluconate. MEMRI experiments were performed at baseline and 7, 14, 21, 28, 35, and 42 min after intraperitoneal injection of Mn (n=4) or Mn-CaG (n=3) into adult male C57B1/6 mice using a 9.4T MRI system. Using a look-locker inversion recovery sequence[2] a series of 12 short-axis gradient echo images were acquired with the following parameters: TE=0.99 ms, TR_{ir} =~3s, TI =~100–1300 ms, FOV=25.6mm^2, DM=128^2, FA=10Ã‚Â°, slice thickness=1.5 mm. Cardiac function was assessed using cine-MRI. For T1 analysis, the average signal intensity in myocardium and blood pool regions were calculated using Segment, and were fitted to an exponential curve.

Results T1 values were evaluated at each time point and compared between two groups. Both manganese-based media rapidly led to significant shortening of T1 in the myocardium and the blood and these changes remained stable over the course of the experiment. The peak reduction of T1 in the myocardium and blood was observed slightly earlier for Mn than for Mn-CaG (figure 1a), although this difference did not reach significance in this pilot study. Image enhancement in cine-MRI was similar in the Mn-CaG group and the Mn group (figure 1b). Importantly no alterations in cardiac function or heart rate were observed and all mice recovered fully, indicating [Mn^{2+}] used was below cardiotoxic levels.

Conclusion The present study indicates that Ca^{2+} supplements to Mn^{2+} does not affect cardiac function while producing an optimum image quality. This approach has the potential of reducing the risk of toxicity of manganese-based agents and could be used to identify salvageable myocardium and monitoring new growth of heart tissue after stem cells therapy.

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159 TRPM2 ION CHANNEL ACTIVATION CONTRIBUTES TO REDOX-SENSITIVE VASCULAR DYSFUNCTION IN HYPERTENSION

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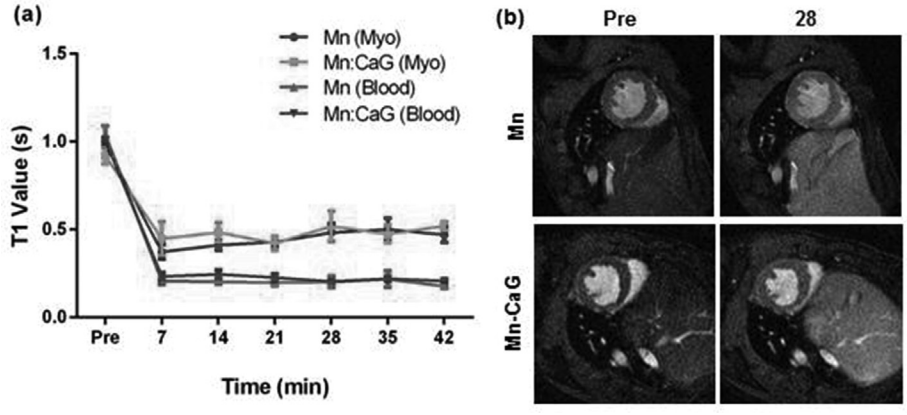
The interplay between reactive oxygen species (ROS) and Ca^{2+} plays a major role in the regulation of vascular function. However, mechanisms underlying ROS-induced Ca^{2+}-influx and signalling are not fully established. The transient receptor potential melastatin 2 cation channel (TRPM2) is a redox-sensitive cation channel that promotes influx of Ca^{2+} after activation by H_2O_2 through PARP-ADPR-dependent mechanisms in inflammatory cells. TRPM2 also regulates Na^+ influx and by increasing intracellular Na^+ content, it could interfere with the function of the Na^+-Ca^{2+} exchanger (NCX), which may confer a novel mechanism whereby ROS influences Ca^{2+} influx and signalling. Here, we postulated that redox-sensitive Ca^{2+} regulation involves TRPM2 and NCX; a process exacerbated in hypertension leading to vascular dysfunction. We also interrogated the role of Nox4 in these processes. Mesenteric arteries from wild-type (WT), LinA3 (chronic Ang II-induced mouse model of hypertension), Nox4$^{-/-}$, and LinA3/Nox4$^{-/-}$ and VSMCs cultures from human arteries were used. Vascular function, assessed by wire myography, demonstrated that mesenteric arteries from LinA3 mice present increased Phe-induced vasoconstriction (Emax – LinA3 vs WT: 9.37±0.51 *vs* 6.79±0.29); an effect ameliorated by olaparib (PARP inhibitor) and 2-APB (TRPM2 blocker). The mRNA expression of NOX4 (fold change: 3.05±0.30), TRPM2 (fold change: 1.38 ±0.24), and NCX exchanger (fold change: 1.97±0.34) were increased in LinA3 mice; an effect not observed in LinA3/Nox4$^{-/-}$ mice (a model with reduced H2O2 levels). Ang II stimulation increased Ca^{2+} influx in human VSMC from normotensive (AUC-Ex490/Em535: 15400±917.5) and hypertensive subjects (AUC-Ex490/Em535: 22460±2388). TRPM2 activation inhibitors, such as 2-APB, olaparib and 8-Br, as well as, NCX inhibitors benzamil, KB-R7943 and YM244769,



Abstract 158 Figure 1 (a) T1 value for myocardium and blood (b) End-diastolic cine-MRI images from before and 28 min after IP injection of Mn or Mn-CaG.