

homeostasis, reactive oxygen species production and, to a lesser extent, ATP generation. The balance of mitochondrial fission, fusion and motility is likely to provide fine control of subcellular location and interactions of the organelle; however, the outcome of perturbation of mitochondrial dynamics on endothelial function remains unclear. We sought to address this gap by investigating the effects of mitochondrial fission inhibitor, Mdivi-1, on endothelial cells.

Treatment of cultured endothelial cells with Mdivi-1 (1 or 10 μ M, 48 hour) increased mitochondrial length and branching extent compared to control, consistent with inhibition of fission. Mdivi-1 increased branched, twisted and looped endothelial mitochondrial morphologies, whilst also reducing net mitochondrial speed. No acute toxicity was observed after Mdivi-1 treatment (10 μ M, 48 hour), however Mdivi-1 did decrease the intracellular content of the glycoprotein von Willebrand Factor (produced, stored and released by endothelial cells to aid thrombosis). Endothelial gap junction communication was also assessed as a function of confluent cells' ability to inter-cellularly transfer the dye, Lucifer yellow; Mdivi-1 decreased dye transfer rates, suggesting reduced intercellular gap junction communication.

In conclusion, Mdivi-1 treatment altered endothelial mitochondrial morphology and dynamics, and also decreased von Willebrand Factor content and gap junction communication, however the mechanistic links remain unclear. Clarification is important, as modulation of mitochondrial dynamics has been proposed as a novel target against the cell proliferation associated with vascular disease.

P27

DEPLETION OF CARDIAC SUCCINATE MEDIATES IMPAIRED HYPOXIA-INDUCIBLE FACTOR 1A SIGNALLING BY LONG CHAIN FATTY ACIDS IN INSULIN RESISTANCE

¹MS Dodd, ¹Mdl Sousa Fialho, ¹CN Montes Aparico, ¹M Kerr, ¹KN Timm, ²JL Griffin, ³JJFP Luiken, ³JFC Glatz, ¹DJ Tyler, ¹LC Heather. ¹Department of Physiology, Anatomy and Genetics, University of Oxford, Parks Road, Oxford, UK; ²Department of Biochemistry, University of Cambridge, Cambridge, UK; ³Department of Molecular Genetics, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands

10.1136/heartjnl-2018-BSCR.32

Hypoxia-inducible factor (HIF)1 α is activated following myocardial infarction, and is critical for cell survival in hypoxia. In cancer, changes in Krebs cycle intermediates have also been shown to affect HIF1 α stabilisation. We questioned whether abnormal metabolism could prevent HIF1 α activation in diabetes. Type 2 diabetic hearts have decreased HIF1 α protein accumulation in ischemia, which correlated negatively with plasma fatty acid (FA) concentrations and positively with myocardial succinate concentrations. In insulin-resistant cardiomyocytes, HIF signalling and downstream metabolic adaptation was suppressed in hypoxia. Impaired HIF1 α stabilisation was due to increased degradation of the protein in hypoxia, as inhibition of the proteasome or inhibition of the HIF hydroxylases was able to increase HIF1 α in insulin resistance. This was due to abnormal metabolism, as FA (both palmitate and oleate) prevented HIF1 α accumulation in a concentration-dependent manner, which could be reversed by blocking CD36 mediated FA uptake. Succinate promotes HIF stabilisation by inhibiting the HIF hydroxylases, however, FA suppressed succinate accumulation during hypoxia. Increasing succinate

concentrations using dimethylfumarate, overrides the FA-mediated inhibition of HIF1 α in a concentration-dependent manner. Pharmacologically inhibiting the HIF hydroxylases promoted HIF1 α accumulation and improved cardiac function following ischemia-reperfusion in diabetic rats. In conclusion, elevated FA in type 2 diabetes prevent HIF1 α accumulation by decreasing succinate concentrations in hypoxia.

P28

IDENTIFICATION OF A SHEAR STRESS RESPONSIVE NOVEL GENE IN THE INTRON OF LAF4

M Ehteramyani, L Yi, Y Hu, L Zeng. Cardiovascular division, James Black Centre, King's College London, London, UK

10.1136/heartjnl-2018-BSCR.33

A novel gene was recently discovered using microarray gene profiling of shear stress-induced genes in mouse embryonic stem cells (ESCs), which is located within the intron of the transcription factor Laf4 and therefore designated as Laf4 intron resident gene (Laf4ir). Laf4 and Laf4ir genes use different strands for transcription. Two transcript variants have been identified for Laf4ir with three potential opening reading frames (ORFs). In this study, we intend to characterise the transcriptional/translational expression, regulation, and functions of this novel gene. The experiments with qRT-PCR analysis revealed that Laf4ir mRNA was unregulated transiently by shear stress compared to static conditions in mouse differentiated ESCs. Laf4ir was expressed in late stage of mouse embryos but not in the early stages and Laf4ir was differentially expressed in adult mice organs/tissues. Specific antibodies against peptides from the ORFs confirmed the translation of these ORFs in mouse differentiated ESCs in response to shear stress. Immunofluorescence staining with antibody against ORF2 revealed that the ORF2 was exclusively expressed in the intima of aorta in wild type mice. Over-expression of ORF2 significantly increased CD31 expression in mouse ESC-derived Sca1+ cells, indicating the potential role of Laf4ir in endothelial cell differentiation. Interestingly, high level of ORF2 was detected from intima to the adventitia of aorta in ApoE^{-/-} mice and in the adventitia of femoral artery from platinum wire injured mice. Further detailed and concrete investigation is required to characterise the potential functions of Laf4ir gene products, which may provide some new insights into vascular biology.

P29

THE TRANSCRIPTION FACTOR BRN-3B/(POU4F2) REGULATES VASCULAR FUNCTION AND INTEGRITY IN VIVO

L Mele, LJ Maskell, VS Budhram-Mahadeo. Medical Molecular Biology Unit, Institute of Cardiovascular Science, University College London, UCL, Rayne Building, London, UK

10.1136/heartjnl-2018-BSCR.34

Rationale Chronic hypertension is a major risk factor for stroke and coronary heart disease. Hypertension is associated with vascular smooth muscle cell (VCMCs) dysfunction, leading to vascular wall calcification and reduced vessel compliance. However, the underlying cellular mechanisms remain unclear. We previously demonstrated that loss of the transcription factor Brn-3b/(POU4F2) results in weight gain and diabetes in mice, known risk factors for hypertension. Herein we present evidences for a direct role of Brn-3b in the maintenance of vascular integrity.

Methodology Blood pressure (pressure-volume analysis or tail cuff plethysmography) was measured in wild type (WT) and Brn-3b knockout (KO) mice. Immunostaining and histological analysis were performed in the aorta of these animals. Gene expression analysis was performed using RNAseq and the expression pattern of Brn-3b was evaluated in primary VSMC cultures by immunofluorescence and qRT-PCR.

Results Brn-3b KO mice (2–6 months) developed spontaneous hypertension and vascular dysfunction such as neointimal hyperplasia, increased extracellular matrix (ECM) deposition and calcification. RNAseq analysis revealed that loss of Brn-3b in the aorta increased ECM gene expression, including collagens. Immunostaining of aortic sections from WT mice showed that Brn-3b was principally expressed in the tunica media, mostly composed of VSMC. Additionally, Brn-3b protein and mRNA were detectable in human and rodent primary VSMC cultures.

Conclusions Brn-3b loss *in vivo* is associated with vascular dysfunction and hypertension. Future studies will aim to investigate the role of Brn-3b in VSMC phenotypical changes.

P30 EFFECTS OF CARNITINE SUPPLEMENTATION IN THE TYPE 1 DIABETIC HEART: AN IN VIVO HYPERPOLARIZED MRS STUDY

Dragana Savic, Kerstin Timm, Vicky Ball, Lisa Heather, Damian Tyler. *Department of Physiology, Anatomy and Genetics, University of Oxford*

10.1136/heartjnl-2018-BSCR.35

Carnitine transports long-chain fatty acids across the mitochondrial membrane for subsequent β -oxidation. It is known that carnitine levels are decreased in cardiac diseases, such as diabetes, and that carnitine supplementation can have cardioprotective effects.

The purpose of this study was to investigate the effects of l-carnitine supplementation on cardiac metabolism in the diabetic rat heart.

Type 1 diabetic rats were generated by streptozotocin injection, control rats were injected with citrate buffer and all were treated for 3 weeks with daily injections of either l-carnitine or saline, where after they were subjected to CINE-MRI and hyperpolarized MRS.

Blood glucose levels were elevated in both diabetic groups, with the saline treated diabetic group showing a progressive increase in hyperglycaemia. Hyperpolarized MRS demonstrated reduction of pyruvate dehydrogenase (PDH) flux in the diabetic groups, but PDH flux was significantly higher in the l-carnitine treated diabetic group. Both lactate and alanine were significantly elevated in the animals treated with l-carnitine.

l-carnitine supplementation stabilises hyperglycaemia and increases the metabolism of pyruvate in the diabetic heart. l-carnitine provides a means to improve pyruvate metabolism in the diabetic heart.

P31 EFFECT OF GLYCOGEN CONTENT ON KETONE BODY OXIDATION AND GLYCOLYSIS IN THE ISOLATED RAT HEART

Azrul Abdul Kadir, Cher-Rin Chong, Henry Lee, Brianna Stubbs, Pete Cox, Kieran Clarke, Rhys Evans. *Department of Physiology, Anatomy and Genetics – University of Oxford*

10.1136/heartjnl-2018-BSCR.36

Apart from acting as an alternative energy source in oxidative tissues, including muscle, ketone bodies regulate the metabolism of other substrates, but are cataplerotic. Glycogen can be mobilised for anaplerosis, regenerating Krebs cycle intermediates, thus glycogen availability may affect the hearts' ability to oxidise ketone bodies. We hypothesise that glycogen acts as an anaplerotic substrate for myocardial ketone body oxidation, and thereby affects cardiac exogenous glucose utilisation. We aimed to determine the effects of cardiac glycogen content and ketone body metabolism on glucose utilisation.

Methods Isolated rat hearts were pre-perfused with buffer containing either no substrate (to deplete glycogen) or pyruvate, lactate, glucose and insulin (to augment glycogen content), before switching to 14C-hydroxybutyrate (β HB) or 5–3 hour-glucose, plus 11 mM glucose. Timed buffer samples were analysed for $^{14}\text{CO}_2$ or $^3\text{H}_2\text{O}$ to measure β HB oxidation or glycolysis, respectively. Hearts were freeze-clamped for glycogen content.

Results Removal of substrate in the perfusion period significantly decreased myocardial glycogen content (5.4 ± 1.6 vs 43.5 ± 5.1 μmol glycosyl units/gww). β HB oxidation rate in high glycogen hearts was twice that of low glycogen hearts. Presence of β HB in both high and low glycogen hearts significantly decreased glycolysis from perfusate glucose by 60% and 38% respectively (both to 0.29 $\mu\text{mol/gww/min}$).

Conclusion β HB oxidation was increased, and glycolysis from exogenous glucose was decreased, in high glycogen hearts.

P32 MITOCHONDRIA MUST CHOOSE BETWEEN RESISTANCE TO FATTY ACYL CoA REGULATION OR RAPID RESPIRATION IN THE TYPE 2 DIABETIC HEART

Matthew Kerr, Sara Rohling, Maria Sousa Fialho, Colleen Lopez, Damian Tyler, Lisa C Heather. *Department of Physiology, Anatomy and Genetics – University of Oxford*

10.1136/heartjnl-2018-BSCR.37

Introduction Cardiac metabolism in type 2 diabetes is abnormal, with increased fatty-acid oxidation, and decreased glucose oxidation. We have previously shown a reduction in energetics in the type 2 diabetic heart (20% decrease in ATP). The control of respiratory rate is mediated primarily (70%) by one protein, the adenine nucleotide translocator (ANT). The ANT is inhibited by fatty acyl CoA groups which may relate the reduced energetics in the type 2 diabetic heart to its high fatty acid environment.

Results Palmitoyl-CoA (P-CoA) decreased respiration in control mitochondria by 50%, but in diabetic mitochondria by just 20%. We showed unchanged V_{max} , but increased K_m in the presence of P-CoA, demonstrating competitive inhibition by the fatty acyl CoA group.

Diabetic mitochondria had decreased ADP stimulated, and maximal respiration compared to controls when respired on glutamate, pyruvate and malate. The addition of fatty acids rescued only the ADP stimulated respiratory defect.

Finally, we showed a very strong correlation between decreased respiration, and decreased sensitivity to fatty acyl CoA regulation. **Conclusion** We have shown that type 2 diabetic mitochondria are resistant to fatty acyl CoA regulation, indicating that this is unlikely to be the cause of energetic dysfunction. We have shown a strong correlation between fatty acid sensitivity and the rate of respiration, two phenomena that are intrinsically linked by the ANT. We propose that changes to ANT kinetics