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T1 ENERGETIC DEFICIENCY AND ADENOSINE RECEPTOR SIGNALLING IN CARDIAC FIBROSIS

[1]A Stockenhuber, [1]S Ghafari, [1]M Kelly, [1]V Steeples, [1]G Czibik, [2]D Tyler, [3]N Støttrup, [1]A Yavari, [1]H Watkins, [1]H Ashrafian. *[1]Radcliffe Department of Medicine, University of Oxford, Oxford, UK; [2]Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK; [3]Department of Clinical Medicine, Aarhus University*

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Myocardial fibrosis (MF) contributes to the pathogenesis of cardiac hypertrophy secondary to energetic perturbation. Recent evidence suggests an increase of purine signalling upon energetic deficiency and specifically a role of adenosine signalling in tissue fibrosis. The specific objective of this study was to delineate adenosine A2A receptors in the development of MF.

In vitro, isolated cardiac fibroblasts demonstrated a significant increase in collagen production upon A2A receptor stimulation. This was inhibited by addition of a specific A2A receptor inhibitor.

In vivo, models of cardiac hypertrophy including the transverse aortic constriction (TAC) model and cardiac actin E99K transgenic mice (E99K mice) were investigated as murine models for MF.

In E99K mice a reduced phosphocreatine/ATP ratio was demonstrated using magnetic resonance spectroscopy. Interstitial adenosine levels measured by microdialysis correlated with collagen content, showing energy deficiency and a correlation with MF. Crossbreeding of E99K and Adenosine A2A receptor knock out (A2A KO) mice resulted in a significant reduction of MF in E99K heterozygous A2A KO animals.

A2A KO mice undergoing TAC demonstrated significantly less fibrosis formation compared to wild type mice upon measurement of myocardial collagen and on histology. This was associated with a significant rescue of cardiac function.

Finally, pharmacologic adenosine A2A receptor inhibition using the antagonist ZM241385 demonstrated a partial rescue effect on MF in both TAC and E99K animals.

This data indicates that signalling of energy deficiency via adenosine A2A receptors play a crucial role in the formation of MF and that this pathway is susceptible to pharmacologic modulation.

T2 FACTOR INHIBITING HIF (FIH1) MODULATES CARDIAC FUNCTION AND METABOLISM

[1]J Holliday, [1]S Platt, [1]MK Handzlik, [1]IA MacDonald, [2]K Clarke, [3]PJ Ratcliffe, [1]MA Cole. *[1]School of Life Sciences, University of Nottingham; [2]Department of Physiology, Anatomy and Genetics University of Oxford; [3]Henry Wellcome Building for Molecular Physiology, University of Oxford*

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Hypoxia-inducible factor (HIF) plays a pivotal role in the cellular response to reduced oxygen availability. HIF activity is regulated by two families of oxygen sensitive enzymes; the prolyl hydroxylase domain (PHD) family, and factor-inhibiting HIF (FIH1). FIH1 is thought to be an essential regulator of metabolism but its role in the heart is unknown.

Mice with a null mutation in the FIH1 gene (FIH1-/-, $n \geq 5$) had 18% lower body weight ($p<0.02$) than wild type littermate controls (WT, $n \geq 6$), but normal total cardiac mass. Right ventricular mass determined via MRI was 25% greater in FIH1-/- hearts ($p<0.01$). Cine MRI revealed a 15% reduction in stroke volume in FIH1-/- hearts, from 27.4 ± 2.3 µl in WT to 23.4 ± 1.5 µl in FIH1-/- ($p<0.05$). Impaired contractility was also observed in individual myocytes (sarcomere shortening was $3.01\% \pm 0.20\%$ in FIH1-/- compared to $3.92\% \pm 0.17\%$ in WT, $p<0.05$) and was associated with reduced Ca2+ transient amplitude (fura-2 ratio 0.21 ± 0.02 and 0.29 ± 0.02 for FIH1-/- and WT respectively, $p<0.05$).

Glycolytic flux (µmol/min/g) was significantly higher in Langendorff perfused FIH1-/- hearts (1.17 ± 0.04) than WT (0.79 ± 0.12, $p<0.05$) although no changes in lactate efflux were detected. There were no differences in pyruvate dehydrogenase kinase 1 and 4 protein expression and citrate synthase activity (µmol/min/mg) was similar for both WT (1.01 ± 0.02) and FIH1-/- (0.96 ± 0.03) hearts.

Our data suggest a novel role for FIH1 in modulating cardiac contractility and metabolism, with FIH1 ablation producing cardiac effects comparable to those associated with activation of the hypoxic signalling pathway.

T3 CARDIAC ARRHYTHMIA RESULTING FROM AN ACCUMULATION OF BRANCHED CHAIN AMINO ACIDS IN A MOUSE LINE WITH A MUTATION IN BCAT2

[1]T Nicol, [2]S Podliesna, [2]V Portero, [1]S Falcone, [1]A Blease, [2]S Casini, [2]CR Bezzina, [2]CA Remme, [1]P Potter. *[1]Disease Model Discovery Group, Mammalian Genetics Unit, MRC Harwell Institute, Oxfordshire, UK; [2]Department of Experimental Cardiology, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands*

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As part of a large-scale phenotype-driven screen we identified a line exhibiting sudden death. Mapping and whole genome sequencing identified a missense mutation in the Bcat2 gene, encoding mitochondrial branched chained aminotransferase, resulting in an early stop (Q300*) and a truncated protein. Homozygous mice exhibited increased plasma and urine levels of branched chain amino acids (BCAAs). Mutations in this pathway have previously been associated with Maple Syrup Urine Disease (MSUD) and can result in neurological symptoms. All homozygous mice died suddenly at 7 weeks of age, without any preceding symptoms. No cardiac abnormalities were observed on histological analysis, nor were there any other significant findings related to MSUD. An accumulation of branched chain amino acids was identified in urine and serum from homozygous mice, but unlike MSUD there was no accumulation of branched chain keto-acids. Homozygous mice showed QTc-prolongation *in vivo* on surface ECG analysis and prolonged action potential duration (APD) *ex vivo* (assessed by optical mapping in isolated hearts). Moreover, isolated hearts from mutant animals displayed increased inducibility of atrial and ventricular arrhythmias. In line with this, patch clamp measurements revealed significant APD90

prolongation and increased incidence of pro-arrhythmic events in isolated cardiomyocytes which was prevented by pharmacological inhibition of the late sodium current. Our current data suggests a direct effect of the mutation on cardiac function rather than the observed phenotypes resulting from an accumulation of BCAAs. Thus we have identified a novel model of sudden cardiac death resulting from abnormal BCAA metabolism.

T4 ERK5 DEGRADATION: A TURNING POINT FROM COMPENSATED METABOLIC CARDIOMYOPATHY TO HEART FAILURE

A Ruiz-Velasco, W Liu, X Wang. *Faculty of Biology, Medicine, and Health, The University of Manchester, Oxford Rd, Manchester, UK*

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Rationale The accumulated prevalence of obesity, diabetes, and metabolic syndrome is more than 25% of the world's population. These are all conditions that have been repeatedly related to a higher risk of heart failure, and effective treatment has not been found. It remains essential to continue deciphering the underlying molecular mechanism to discover novel treatment strategies.
Methodology Initial screening was performed on myocardium samples from ob/ob mice, db/db mice, rhesus monkey with spontaneous metabolic syndrome, and mice fed for 25 weeks with high-fat diet (HFD). In subsequent studies, extracellular signal-regulated protein kinase 5 (ERK5) cardiomyocyte-specific knockout mice (ERK5-cko) were evaluated up until 16 weeks of HFD feeding. *In vitro* experiments were performed on rat ventricular myocytes treatd with saturated fatty acids.
Results The screening of obese and diabetic models showed that ERK5 was selectively lost in the myocardium. ERK5-cko presented cardiac dysfunction after only 16 weeks of HFD. Further studies showed the loss of contractility was accompanied by augmented oxidative stress, increased lipid accumulation, and severe mitochondrial dysfunction. Mechanistic studies revealed ERK5 to act upstream of the mitochondrial regulator peroxisome proliferator-activated receptor γ co-activator-1α (PGC-1α). Moreover, it was observed that ERK5 degradation after saturated fatty acid treatment was mediated by calpain-1, while the inhibition of this degradation could prevent the mitochondrial dysfunction.
Conclusion The calpain-mediated degradation of ERK5 blunts the compensatory response that would usually maintain mitochondrial integrity when facing metabolic stress.

T5 VERY LOW CALORIE DIET IN OBESITY IMPROVES METABOLIC RISK FACTORS AT THE INITIAL COST OF VENTRICULAR FUNCTION AND STEATOSIS

JJ Rayner, MA Peterzan, S Neubauer, OJ Rider. *OCMR, Division of Cardiovascular Medicine, University of Oxford*

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Background Very Low Calorie Diets (VLCD) in obesity are an effective weight loss intervention that rapidly reduce liver fat and improve peripheral insulin resistance. We hypothesised that improved peripheral glycaemic control would be accompanied by initial impairment of cardiac function as hepatic fat stores are mobilised and taken up by the myocardium.
Methods 14 obese volunteers (4 male, 49±15 years, BMI 36.2±5.9 kg m-2) underwent body composition analysis and MR scanning for abdominal visceral and liver fat, LV structure and function, 1H-MRS to measure myocardial triglyceride content (MTGC), and echocardiography for diastolic function (E/E'), before and one week into a VLCD (800 kcal/day).
Results 7 days of VLCD led to significant reductions in total body fat, visceral and hepatic fat, and insulin resistance. However, MTGC rose from 1.74±0.99% to 3.02±1.70% (p=0.030), and there was a reduction in both systolic function (LVEF 67±3% to 62±5%, p=0.014; peak radial strain 51 ±8% to 42±9%, p=0.005) and diastolic function (e/e' 8.5 ±1.6 to 10.3±3.5, p=0.034). The change in MTGC at one week correlated with change in diastolic function (r=0.729, p=0.017). However at 8 weeks (n=6), changes in MGTC as well as cardiac function had returned to normal.
Conclusions We demonstrate for the first time in healthy obese individuals that a 7 day period of severe caloric restriction results in accumulation of myocardial fat which is associated with impairment of both systolic and diastolic LV function at this timepoint, despite a significant improvement in hepatic fat and whole body insulin sensitivity.

P1 CAUSAL LINK BETWEEN INTRACELLULAR SODIUM OVERLOAD AND METABOLIC REMODELLING IN THE HEART: UNCOUPLING ATP SUPPLY AND DEMAND?

[1]D Aksentijević, [2]A Karlstädt, [1]M Basalay, [1]BA O'Brien, [3]A Thakker, [3]D Tennant, [4]W Fuller, [2]H Taegtmayer, [5]TR Eykyn, [1]MJ Shattock. *[1]British Heart Foundation Centre of Research Excellence, King's College London, The Rayne Institute, St Thomas' Hospital, London, UK; [2]Department of Internal Medicine, Division of Cardiology, The University of Texas Health Science Centre at Houston, McGovern Medical School, Houston, Texas, USA; [3]Institute of Metabolism and Systems Research, College of Medical and Dental Sciences University of Birmingham, Edgbaston, Birmingham, UK; [4]Division of Cardiovascular and Diabetes Medicine, Medical Research Institute, College of Medicine, Dentistry and Nursing, University of Dundee, Ninewells Hospital and Medical School, Dundee, UK; [5]Department of Imaging Chemistry and Biology, Division of Imaging Sciences and Biomedical Engineering, King's College London, St Thomas' Hospital, London, UK*

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Rationale Intracellular Na elevation is a hallmark of the ischaemic and failing heart – pathologies in which both acute and chronic metabolic remodelling occur.
Objective To assess whether acute (75 μM ouabain 100 nM blebbistatin) and chronic myocardial Nai load (PLM3SA mouse) are causally linked to metabolic remodelling and if the failing heart shares a common Na-mediated metabolic 'fingerprint'.
Methods 23Na, 31P and 13C NMRS were performed in normal and hypertrophied (pressure overload) Langendorff perfused mouse hearts followed by 1 hour NMRS metabolomic profiling, mass spec and *in silico* modelling.
Results Na overload (acute, chronic (PLM3SA), and hypertrophy 2, 1.3 and 1.4-fold respectively) resulted in common metabolic perturbations: substrate switch (palmitate 35% reduction, glucose 58% increase), flux (TCA cycle, OXPHOS, glycolysis) and metabolomic profile (TCA cycle, glycolysis, anaplerosis) without energetic impairment (PCr/ATP 1.5 ±0.1 vs control 1.3±0.1). Inhibition of mitochondrial Na/Ca exchanger by CGP37157 during both acute and chronic Na load ameliorated the metabolic changes.