

Myocardial energetics and redox in health and disease

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001 SUBSTRATE UTILISATION BY THE FAILING HUMAN HEART BY DIRECT QUANTIFICATION USING ARTERIOVENOUS BLOOD SAMPLING

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Metabolic substrate utilisation of the human failing heart is an area of controversy. The purpose of this study is directly to quantify myocardial substrate utilisation in moderately severe heart failure, type 2 diabetes and healthy controls using simultaneous coronary sinus and arterial blood sampling. Patients with heart failure (n=9, mean NYHA 2.7 \pm 0.5), with type 2 diabetes (n=5) and with normal heart function (n=10) were studied after an overnight fast in connection with electrophysiological investigations/treatments. A systemic infusion of [2H₂] palmitate allowed for the calculation of absolute palmitate extraction across the heart. Blood samples were analysed for non-esterified fatty acids, triacylglycerol, glycerol, glucose, pyruvate, lactate, 3-hydroxybutyrate and blood gases after simultaneous sampling of arterial and coronary sinus blood. Arteriovenous sinus metabolite concentration differences and fractional extractions for all substrates were similar between the groups. The absolute non-esterified fatty acid uptakes assessed by [2H₂] palmitate extraction were also similar between the groups. Using direct measurements of metabolic substrate uptake by arteriovenous difference technique, the compensated human failing heart does not appear to have reduced myocardial fatty acid uptake.

002 COMPENSATION FOR IMPAIRED MYOCARDIAL PHOSPHOTRANSFER IN GUANIDINOACETATE-N-METHYLTRANSFERASE KNOCKOUT MICE

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Guanidinoacetate-N-methyltransferase (GAMT) is a key enzyme in creatine biosynthesis, such that GAMT knockout mice lack phosphocreatine (PCr) as a substrate for energy transfer via the creatine kinase (CK) reaction. Despite undetectable levels of PCr and creatine, GAMT knockout mice exhibit only minor changes in baseline function and impaired contractile reserve, suggesting remarkable plasticity in myocardial energy metabolism. However, the precise nature of compensatory metabolic mechanisms remains unknown. The aim of this study was to examine the potential roles of F1F0 ATP synthase and the complementary phosphotransfer enzyme adenylate kinase in GAMT knockout hearts. Mitochondria were isolated from the 27-week GAMT knockout and age-matched wild-type hearts. To assay the F1F0 ATP synthase capacity, maximal F1 ATPase hydrolytic activity was measured spectrophotometrically in

mitochondrial homogenate by coupling ATP hydrolysis to NADH oxidation. Total enzyme activities of CK, adenylate kinase and glycolytic enzymes (glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, pyruvate kinase) were measured in ventricular tissue extracts using coupled enzyme assays. GAMT knockout hearts were characterised by a marked increase in F1F0 ATP synthase activity (oligomycin sensitive activity 2.16 \pm 0.5 vs 4.2 \pm 1.1 μ mol ATP/minute per mg; n=6, p<0.05), decreased CK (6.8 \pm 0.6 vs 5.0 \pm 0.4 U/mg, p<0.01; n=13), and unaltered adenylate kinase activity (2.5 \pm 0.6 vs 2.6 \pm 0.6 U/mg; n=11), while glycolytic enzyme activities were consistently elevated in knockout hearts. Therefore, long-term adaptation to chronic perturbation of the CK/PCr system in GAMT knockout hearts does not include a compensatory increase in phosphotransfer via adenylate kinase. Rather, this study suggests increased ATP synthesis as a potential compensatory mechanism to maintain cardiac function close to normal.

003 REAL-TIME ASSESSMENT OF KREBS CYCLE METABOLISM WITH HYPERPOLARISED [2-13C]PYRUVATE

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Background and Objectives The Krebs cycle is fundamental to cardiac energy production, and is often implicated in energetic imbalances characteristic of heart disease. To date, Krebs cycle flux has been measured using ¹³C-magnetic resonance spectroscopy with isotopomer analysis; however, this approach is limited to the study of steady-state metabolism only and has limited in-vivo applications. The aim of this work was to assess the feasibility of using hyperpolarised [2-¹³C]pyruvate as a metabolic tracer to monitor real-time Krebs cycle metabolism directly in vivo.

Methods [2-¹³C]Pyruvate was hyperpolarised and dissolved to form an 80 mM solution, 1 ml of which was injected over 10 s via a tail vein catheter into an anaesthetised rat positioned in a 7T magnetic resonance scanner. Spectra were acquired for 1 minute following injection with 1 s temporal resolution. The signal was localised to the heart using a surface coil.

Results Peaks arising from hyperpolarised [2-¹³C]pyruvate were identified as citrate, glutamate, acetyl-carnitine, lactate and alanine using phantom experiments and ¹H-¹³C correlation nuclear magnetic resonance spectroscopy of tissue extracts. Identified peaks visible with 1 s resolution were analysed.

Conclusions This result demonstrates the first example of direct monitoring of instantaneous Krebs cycle metabolism in vivo. The entry of [2-¹³C]pyruvate into the Krebs cycle has been monitored with 1 s temporal resolution. Future experiments utilising hyperpolarised [2-¹³C] pyruvate in a variety of pathological and physiological conditions will undoubtedly provide useful insights into the mechanisms driving energetic imbalances often expressed in heart disease.

004 PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA IS ESSENTIAL FOR CARDIAC ADAPTATION TO CHRONIC HYPOXIA

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Deletion of the peroxisome proliferator-activated receptor alpha (PPAR α) gene in mice results in abnormal cardiac substrate metabolism and PPAR α -/- hearts have impaired function at high workload and increased post-ischaemic infarct size. We hypothesised that PPAR α -/- mouse hearts would be intolerant to chronic hypoxia,

and that PPAR α is an essential regulator of metabolism in hypoxia. PPAR α -/- mice (n=18) and wild-type (wt) controls (n=20) were exposed to 3 weeks of normobaric hypoxia. Control activated receptor alpha-/- (n=17) and wt mice (n=21) were housed in normoxic conditions within the same room. Oxygen content was reduced incrementally in the first week of housing, followed by 2 weeks at 11% oxygen. In-vivo cardiac function was measured using multislice cardiac magnetic resonance imaging. Hearts were perfused in the Langendorff mode to measure palmitate oxidation and glycolysis using 3H-labelled substrates. Cardiac output was unchanged in hypoxic wt and normoxic PPAR α -/- mice, but was reduced by 31% by hypoxia in PPAR α -/- mice (p<0.02). Late-stage ventricular filling was 46% lower in hypoxic PPAR α -/- mice (p<0.01). Hypoxia reduced palmitate oxidation by 27% in mouse wt hearts, but did not affect PPAR α -/- hearts. Hypoxia increased net lactate efflux 2.4-fold in hearts from wt animals (p<0.01), but lactate efflux from PPAR α -/- hearts was unchanged with hypoxia. Hypoxia increased basal glycolytic flux 2.4-fold in wt hearts but did not alter glycolytic flux in PPAR α -/- mouse hearts (p<0.01), which was already 3.7-fold greater than wt hearts. Thus PPAR α -/- hearts lack the metabolic flexibility essential for adaptation to chronic hypoxia, and their inability to upregulate glycolysis probably impairs cardiac function.

005 NOX2-DEFICIENT BONE MARROW-DERIVED MACROPHAGES EXHIBIT DEFECTS IN CELL SPREADING AND MIGRATION

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Introduction Our previous studies have demonstrated that the Nox2 isoform of NADPH oxidase is essential for the development of cardiac fibrosis in response to either angiotensin II or aldosterone. These in-vivo studies could not, however, identify the critical cell type responsible for this protective effect. As monocytes/macrophages are known to play an important role in the initiation of fibrosis, the current studies investigated whether Nox2 influences the ability of macrophages to migrate in response to the chemo-attractant, colony stimulating factor-1 (CSF-1).

Methods CSF-1-dependent bone-marrow derived macrophages (BMM) were isolated from femurs of wild-type (wt) and Nox2 knockout (Nox2^{-/-}) mice. BMM were seeded onto glass coverslips, starved of CSF-1, re-stimulated with CSF-1, fixed and stained for F-actin. Cell images were analysed for shape (elongation) and spread area using Image J software. Mean cell speed and chemotactic potential in a CSF-1 gradient were analysed using the Dunn Chemotaxis chamber.

Results A number of endpoints were assessed both under growing (basal) conditions and following CSF-1 stimulation. Under growing conditions Nox2^{-/-} BMM had a significantly increased spread area (proportion of the cell in contact with the glass coverslip) compared with wt cells (0.261±0.03 vs 0.237±0.03; p<0.05). However, while CSF-1 stimulation increased the spread area in wt cells above growing conditions (0.261±0.01) Nox2^{-/-} BMM returned only to their initial spread area (0.256±0.01). Nox2^{-/-} BMM were, however, significantly more elongated than wt cells following CSF-1 stimulation (0.670±0.01 vs 0.624±0.01). Importantly, Nox2^{-/-} BMM had a significantly reduced migration speed (0.46±0.03 vs 0.6116±0.02 μ m/minute, p<0.0004), a significant reduction in their persistence of direction (0.15±0.02 vs 0.38±0.02 p<<0.0005) and failed to migrate positively towards CSF-1. Nox2^{-/-} cells also had significantly lower increases in Akt and ERK1/2 phosphorylation following CSF-1 stimulation compared with wtT cells.

Conclusions Nox2^{-/-} BMM display marked abnormalities in morphological and migratory behaviour that may contribute significantly to the ability of the monocyte to differentiate and migrate in vivo in response to pathological stimuli. This phenotype could underlie the protection against fibrosis observed in vivo in Nox2^{-/-} mice.

006 NORMOBARIC HYPOXIA IMPAIRS CARDIAC ENERGETICS IN NORMAL HUMAN VOLUNTEERS

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Background In the first few days of hypoxic exposure, left ventricular dysfunction is consistently observed in the human heart, yet the cellular mechanisms underlying the dysfunction are poorly understood.

Objective Our hypothesis was that normobaric hypoxia impairs cardiac energetics, leading to cardiac dysfunction in healthy subjects.

Methods Normal healthy volunteers underwent 20 h of normobaric hypoxia in purpose-built hypoxia chambers. The partial pressure of oxygen during end tidal expiration (PETO₂) was kept between 50 and 60 mm Hg, while keeping peripheral oxygen saturation (SpO₂) above 80%. Cardiac function was measured using magnetic resonance imaging and echocardiography. High-energy phosphate metabolism was measured as the ratio of phosphocreatine to ATP (PCr/ATP) by ³¹P phosphorus magnetic resonance spectroscopy before and after 20 h of hypoxia. Healthy men (n=12, aged 24±2 years) were recruited from the University of Oxford.

Results During hypoxia, PETO₂ and SpO₂ averaged 55±1 mm Hg and 83.6±0.4%, respectively. There was a 15% reduction in cardiac PCr/ATP, from 2.0±0.1 to 1.7±0.1 after hypoxia (p<0.01) and reduced diastolic function, measured as E/E', from 6.1±0.4 to 7.5±0.7, p<0.01.

Conclusion Short-term normobaric hypoxia led to rapid changes in cardiac metabolism and alterations in diastolic function in normal human hearts. Impaired high-energy phosphate metabolism may explain the cardiac dysfunction observed after hypoxic exposure, whether in health or disease.

007 ROLES OF P47PHOX S303/S304 PHOSPHORYLATION IN TNF α -INDUCED ENDOTHELIAL REACTIVE OXYGEN SPECIES PRODUCTION AND MITOGEN-ACTIVATED PROTEIN KINASE ACTIVATION

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Endothelial cells express constitutively a Nox2 oxidase, which by generating reactive oxygen species (ROS) plays an important role in TNF α signalling. The Nox2 has at least four regulatory subunits and p47phox is a major regulatory subunit of this enzyme. It has been reported that phosphorylation of double serines (S303/S304) in p47phox is a key step for Nox2 activation. In this study, we investigated the role of p47phox S303/S304 phosphorylation in TNF α -induced ROS production and mitogen-activated protein kinase (MAPK) activation in endothelial cells. Serines 303/304 (human p47phox cDNA) were replaced to alanines by site-directed mutagenesis and the wild-type and mutated p47phox were used to transfect a mouse microvascular endothelial cell line (SVEC4-10). Forty-eight hours after transfection, cells were stimulated with or