

## Myocardial energetics and redox in health and disease

Monday 7th and Tuesday 8th September  
2009, University of Oxford

Organisers: Barbara Casadei, Kieran Clarke and  
Saadeh Suleiman

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

doi:10.1136/hrt.2009.191049a

<sup>1,3</sup>J Funada, <sup>2</sup>T R Betts, <sup>1</sup>L Hodson, <sup>1</sup>S M Humphreys, <sup>2</sup>J Timperley, <sup>1</sup>K N Frayn, <sup>1,4</sup>F Karpe. <sup>1</sup>The Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, UK; <sup>2</sup>The Department of Cardiology, John Radcliffe Hospital, Oxford, UK; <sup>3</sup>The Department of Cardiology, NHO Ehime National Hospital, Ehime, Japan; <sup>4</sup>NIHR Oxford Biomedical Research Centre, Oxford Radcliffe Hospital Trust, Oxford, UK

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±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

doi:10.1136/hrt.2009.191049b

D Aksentijevic, C Lygate, K Mäkinen, L Sebag-Montefiore, D Medway, S Neubauer. Department of Cardiovascular Medicine, University of Oxford, Henry Wellcome Building of Genomic Medicine, Roosevelt Drive, Oxford, UK

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±0.5 vs 4.2±1.1 μmol ATP/minute per mg; n=6, p<0.05), decreased CK (6.8±0.6 vs 5.0±0.4 U/mg, p<0.01; n=13), and unaltered adenylate kinase activity (2.5±0.6 vs 2.6±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

doi:10.1136/hrt.2009.191049c

H Atherton, M Schroeder, M Dodd, D Ball, J Griffin, K Clarke, G Radda, D Tyler. Cardiac Metabolism Research Group, Department of Physiology, Anatomy and Genetics, University of Oxford, UK

**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 <sup>13</sup>C-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-<sup>13</sup>C]pyruvate as a metabolic tracer to monitor real-time Krebs cycle metabolism directly in vivo.

**Methods** [2-<sup>13</sup>C]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-<sup>13</sup>C]pyruvate were identified as citrate, glutamate, acetyl-carnitine, lactate and alanine using phantom experiments and <sup>1</sup>H-<sup>13</sup>C 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-<sup>13</sup>C]pyruvate into the Krebs cycle has been monitored with 1 s temporal resolution. Future experiments utilising hyperpolarised [2-<sup>13</sup>C] 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

doi:10.1136/hrt.2009.191049d

M Cole, A Abd Jamil, E Sutton, L Heather, D Stuckey, A Murray, R Evans, K Clarke. Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK

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