and that PPARα is an essential regulator of metabolism in hypoxia. PPARα−/− 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 PPARα−/− mice, but was reduced by 31% by hypoxia in PPARα−/− mice ($p<0.02$). Late-stage ventricular filling was 46% lower in hypoxic PPARα−/− mice ($p<0.01$). Hypoxia reduced palmitate oxidation by 27% in mouse wt hearts, but did not affect PPARα−/− hearts. Hypoxia increased net lactate efflux 2.4-fold in hearts from wt animals ($p<0.01$), but lactate efflux from PPARα−/− hearts was unchanged with hypoxia. Hypoxia increased basal glycolytic flux 2.4-fold in wt hearts but did not alter lycolytic flux in PPARα−/− mouse hearts ($p<0.01$), which was already 3.7-fold greater than wt hearts. Thus PPARα−/− 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

doi:10.1136/hrt.2009.191049e

[1]S Chaubey, [2]C M Wells, [3]G E Jones, [1]A M Shah, [1]A C Cave. *[1]King' College London BHF Centre of Excellence, Cardiovascular Division, London, UK; [2]King's College London, Division of Cancer Studies, London, UK; [3]King's College London, Randell Division of Cell and Molecular Biophysics, London, UK*

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 chemoattractant, 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 μm/minute, $p<0.0004$), a significant reduction in their persistence of direction (0.15±0.02 vs 0.38±0.02 $p\ll0.0005$) 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

doi:10.1136/hrt.2009.191049f

[1,2]C Holloway, [1,2]L Cochlin, [1,2]I Codreanu, [1]E Bloch, [1]M Fatemiah, [2]C Szmigielski, [1]H Atherton, [1]L Heather, [2]J Francis, [2]S Neubauer, [1]P Robbins, [1]K Clarke. *[1]Department of Physiology, Anatomy and Genetics, University of Oxford, UK; [2]University of Oxford Centre for Clinical Magnetic Resonance Research, John Radcliffe Hospital, Oxford, UK*

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 ($PETO_2$) was kept between 50 and 60 mm Hg, while keeping peripheral oxygen saturation (SpO_2) 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 31phosphorus 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, $PETO_2$ and SpO_2 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 TNFα-INDUCED ENDOTHELIAL REACTIVE OXYGEN SPECIES PRODUCTION AND MITOGEN-ACTIVATED PROTEIN KINASE ACTIVATION

doi:10.1136/hrt.2009.191049g

L Teng, J-M Li. *Cardiovascular Research Theme, Faculty of Health and Medical Sciences, University of Surrey, UK*

Endothelial cells express constitutively a Nox2 oxidase, which by generating reactive oxygen species (ROS) plays an important role in TNFα 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 TNFα-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