

Real-time assessment of the effect of acute and chronic hypoxia on cardiac metabolism in the control and diabetic rat: an *in vivo* study

Lydia Le Page¹, Oliver Rider², Victoria Noden¹, Andrew Lewis², Latt Mansor¹, Lisa Heather¹, and Damian Tyler¹

¹Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom, ²Oxford Centre for Clinical Magnetic Resonance Research, Oxford, United Kingdom

Background: Currently over 340 million people worldwide suffer from type 2 diabetes¹, and as such it is crucial to understand the underlying aetiology of the disease, of which hypoxia may be a key aspect. Diabetics suffer microvascular complications, leading to reduced perfusion and oxygen delivery, and also have poorer prognosis after myocardial infarction², potentially due to compromised hypoxic signalling³. Research into the metabolic response of diabetics to hypoxia may provide a new perspective for therapies.

Aim: This study aimed to investigate *in vivo*, real-time cardiac metabolism of both control and diabetic rats, subjected to either an acute hypoxic insult or a chronic hypoxic environment.

Methods:

Model Development - Male Wistar rats were fed a high-fat diet (comprising of 60% fat) for 3 weeks in combination with a single low-dose intraperitoneal (i.p) injection of STZ to generate a model of type II diabetes⁴.

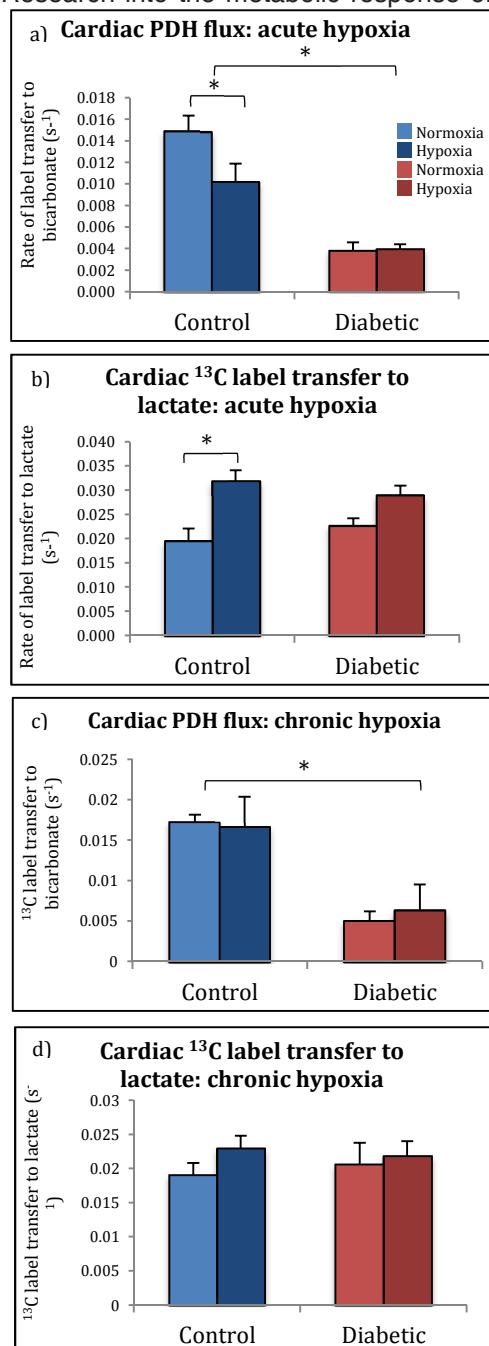
Hypoxic Methodology - The acute hypoxic insult (30 minutes) was performed by altering the O₂:N₂ ratio of the delivered isoflurane anaesthesia to achieve a blood oxygen saturation level equivalent to 11% inspired oxygen (n=8 control, n=8 diabetic). The chronic hypoxic environment was achieved by housing animals in 11% oxygen for 2 weeks, following a week of adaptation (n=8 control normoxia, n=8 control hypoxia, n=8 diabetic normoxia, n=8 diabetic hypoxia). Animals were subsequently anaesthetised at the relevant oxygen concentration during ¹³C spectral acquisition.

Data Acquisition – All animals were anaesthetised and placed in a 7 T MRI system. Approximately 30 mg of [1-¹³C]pyruvic acid doped with 15 mM trityl radical (OX063, GE Healthcare) and a trace amount of Dotarem (Guerbet, France) was hyperpolarized with 30 min of microwave irradiation. The sample was subsequently dissolved in a pressurised and heated alkaline solution, containing 100 mg/L EDTA. This mixture yielded a solution of 80 mM hyperpolarized sodium [1-¹³C]pyruvate with a polarization of ~30% at physiological temperature and pH, of which 1 ml was administered to the animal over 10 seconds via a tail vein catheter⁵. Sixty ¹³C spectra obtained over one minute enabled assessment of cardiac metabolism, using production of ¹³C bicarbonate as a measure of pyruvate dehydrogenase (PDH) flux.

Results: Acute hypoxia caused a reduction in PDH flux, and a significant elevation of ¹³C lactate in control animals, neither of which was replicated in the diabetic group (Figures a and b). A reduced diabetic cardiac PDH flux compared to controls was observed in all experiments, however chronic hypoxia caused no further changes in either PDH flux or ¹³C lactate production (Figures c and d). Hypoxia caused elevated plasma lactate in both groups; expression of hypoxia-responsive proteins GLUT1 and PDK1 was unchanged.

Conclusion: Control animals were able to respond to the altered redox state induced by acute hypoxia, whereas diabetics were not. These data indicate a metabolic inflexibility in the diabetic heart, which may contribute to a worse response to a hypoxic insult such as myocardial infarction. In contrast, PDH flux was not affected by chronic hypoxia in either the control or diabetic heart, which we propose was due to physiological adaptations to the hypoxic environment over the three weeks, removing the stimuli for metabolic changes.

References: ¹ WHO 2013. ²Marfella *et al.*, Diabetes, 2004. ³Heather *et al.*, J. Mol. Cell. Cardiol., 2011. ⁴Mansor *et al.*, Cardiov. Diabetology, 2013. ⁵Schroeder *et al.*, PNAS, 2008



Assessing cardiac metabolism by injection of hyperpolarized pyruvate *in vivo* in both control and diabetic animals. Studies looked at acute hypoxia: a) effect on PDH flux and b) effect on ¹³C label transfer to lactate, and chronic hypoxia: c) effect on PDH flux and d) effect on ¹³C label transfer to lactate.