THE USE OF FATTY ACIDS WITH HYPERPOLARIZED PYRUVATE TO STUDY CARDIAC SUBSTRATE METABOLISM IN THE ISOLATED PERFUSED HEART

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Introduction: The isolated perfused heart provides an ideal model system to investigate cardiovascular disease due to its reproducibility, and its ability to yield metabolic and physiological information independent of the variables found *in vivo*. When combined with the recently developed technique of Dynamic Nuclear Polarization (DNP), the perfused heart can provide useful metabolic information through the application of hyperpolarized ^{13}C labelled metabolites, particularly ^{13}C pyruvate[1]. To ensure that a perfused heart preparation is as physiological as possible, fatty acids should ideally be provided in the perfusion buffer[2]. However, previous work has shown that the method of using bovine serum albumin (BSA) to solubilise long chain fatty acids (FA) significantly reduces the observed signal from hyperpolarized pyruvate due to binding of the pyruvate by albumin[3]. The aim of this work was therefore to find alternative ways of providing the perfused heart with a source of FA, without compromising the hyperpolarized signal intensity. To achieve this goal, we provided the perfused heart with either Intralipid (a triglyceride emulsion) or butyrate (a short chain, soluble FA) and assessed the effect of these fatty acid sources on the signal from hyperpolarized pyruvate. The metabolic and energetic effects of these different fatty acid sources were also assessed.

Materials and Methods:

Heart perfusion: Hearts from 10 male Wistar rats (Harlan, UK) in two groups (Intralipid perfusion N = 5, butyrate perfusion N = 5) were perfused in the Langendorff mode using Krebs Henseleit (KH) buffer oxygenated with 95% O_2/5% CO_2. A polyethylene balloon was placed into the left ventricle in order to measure contractile function. The hearts were placed in the bore of an 11.7 T MRI system (Bruker-Biospin, Germany) for spectral assessment.

Spectroscopy: The hearts were initially perfused with KH buffer containing only 10 mM glucose and 2.5 mM pyruvate. After locating the heart in the centre of the magnet and shimming to reduce the proton linewidth to less than 50 Hz, a hyperpolarized pyruvate study was performed as previously described[1]. Briefly, a 2.5 mM hyperpolarized [1-^{13}C]pyruvate solution was delivered to the heart as a bolus over 120 seconds, and a ^{13}C pulse-acquire experiment commenced (TR = 1 s, FA = 30°, BW = 180 ppm, 8192 pts). Following acquisition, the ratio of the maximum bicarbonate signal to the maximum pyruvate signal was used as a measure of flux through the pyruvate dehydrogenase (PDH) enzyme. The KH buffer was subsequently switched to one containing glucose (10 mM), pyruvate (2.5 mM) and either Intralipid (0.4 mM) or butyrate (0.4 mM) and following a period of stabilization (~30 minutes) a repeat hyperpolarized pyruvate dissolution was carried out. Throughout the protocol, contractile function was recorded from the polyethylene balloon inside the left ventricle and ^{31}P spectroscopy was performed to assess cardiac energetics.

Results and Discussion: No significant change in the energetic or mechanical function of the heart was seen on switching to a perfusion buffer containing either Intralipid or butyrate. There was also no decrease in the intensity of the hyperpolarized pyruvate signal in the presence of either fatty acid source. However, perfusion with both Intralipid (0.4 mM) and butyrate (0.4 mM), led to a decreased in observed bicarbonate production (a measure of PDH flux) when compared to perfusion with glucose and pyruvate alone (Figure 1). This provides direct verification of the glucose-fatty acid (Randle) cycle, whereby the metabolism of fatty acids leads to the inhibition of carbohydrate utilization. The successful uptake and utilization of Intralipid and butyrate by the myocardium was further demonstrated by radiolabelled triglyceride and hyperpolarized butyrate[4] experiments, respectively.

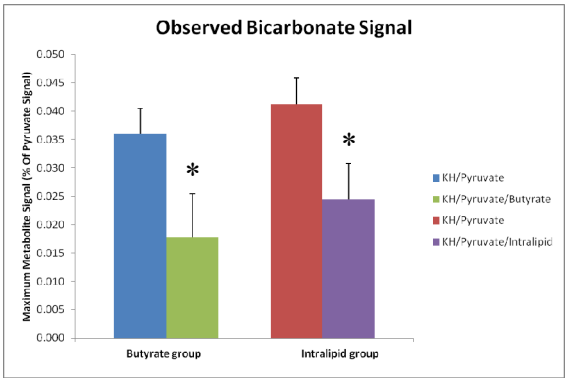

Figure 1: The observed hyperpolarized bicarbonate signal seen when using pyruvate alone or pyruvate plus butyrate or Intralipid. * P<0.05

Conclusion: This work has demonstrated two potential alternatives to the use of BSA to successfully provide the perfused heart with a source of fatty acids without affecting the integrity of the hyperpolarized pyruvate experiment. Whilst butyrate does not fully represent the oxidative pathways of a long chain fatty acid, it does prove successful at providing the heart with a source of fuel in the form of a fatty acid. The use of Intralipid in the perfusion buffer would appear to provide a satisfactory source of fatty acids to allow for more physiological experiments when using hyperpolarized pyruvate in the setting of the perfused heart model.

[1] [Schroeder MA et al, FASEB J. 2009 Aug;23(8):2529-38], [2] [Taegtmeyer H et al, Biochem J. 1980 Mar 15;186(3):701-11], [3] [Moreno KX et al, Am J Physiol Heart Circ Physiol. 2010 May;298(5):H1556-64], [4] [Ball DR et al, ISMRM 2011 abstract 203]

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