The Metabolic Effects of Pyruvate Infusion during Hyperpolarized Magnetic Resonance Experiments

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Introduction

The ability to hyperpolarize ^{13}C-labelled substrates and visualise their *in vivo* uptake and metabolism using magnetic resonance spectroscopy (MRS) has provided a powerful new technique for studying the metabolic changes associated with disease. Hyperpolarized MR experiments to date have focussed on [1-^{13}C]pyruvate metabolism in a range of physiological and pathological states [1]. Biologically, pyruvate is an important molecule to study as deranged cellular substrate utilisation has been identified as a key characteristic of many diseases and pyruvate metabolism is an important regulatory point in cellular substrate selection and energy generation. Furthermore, pyruvate is an ideal molecule for analysis by hyperpolarized MRS as it polarizes well, it has a long longitudinal relaxation time (T_1), and upon injection it is readily taken up by tissues and rapidly metabolised into compounds of biological interest [1].

Typically, in order to assess pyruvate metabolism in rats, 1 ml of 80 mM hyperpolarized [1-^{13}C]pyruvate is injected intravenously [2]. Although upon injection this will be diluted in the bloodstream and rapidly taken up by multiple tissues, it is likely that the resulting plasma concentration of pyruvate will be significantly higher than the resting physiological concentration of approximately 80 µM, and this in turn may affect systemic metabolism. Therefore the aim of this study was to monitor the plasma concentration of pyruvate and other important metabolites to assess the effects of injecting supra-physiological concentrations of pyruvate during hyperpolarized MRS experiments.

Methods

Male Wistar rats (~250 g, n = 14) were anaesthetised. A 2 cm ventral skin incision was made along the crease formed by the abdomen and the left thigh, and the adductor muscles were blunt dissected to visualise the left femoral artery. A small incision was made into the artery and a cannula was inserted into the vessel and secured in place with suture. A second cannula was inserted into the tail vein for pyruvate infusion. Approximately 0.5 ml of blood was sampled from the femoral artery for baseline metabolite measurements. One millilitre of 80 mM sodium pyruvate was then infused, via the tail vein, and further 0.5 ml blood samples were collected at 30 s, 1 min, 2 min, 5 min, 10 min and 30 min post infusion. A 50 µl blood sample was immediately extracted using 7% ice cold perchloric acid. All samples were centrifuged (3,400 rpm for 10 min at 4 ºC), and plasma was removed. A 50 µl aliquot of plasma was separated and 1 µl tetrahydrolipstatin (THL, 30 µg/ml) was added for non-esterified fatty acid (NEFA) analysis. The perchloric acid treated plasma was used to determine pyruvate concentrations spectrophotometrically using a pyruvate assay kit (Biovision). An ABX Pentra 400 (Horiba ABX Diagnostics) was used to perform plasma assays for glucose, lactate, β-hydroxybutyrate, NEFA and TAG, and insulin was measured using a rat insulin ELISA (Mercodia).

Results

The results of this study are summarised in Figures 1 and 2. At baseline, the pyruvate concentration was found to be 60 ± 20 µM, and increased to 253 ± 40 µM at 1 min post 1 ml infusion of 80 mM sodium pyruvate (Figure 1). By 30 min, plasma pyruvate levels were no longer significantly different from baseline values (P= 0.3, Figure 1).

The circulating concentrations of glucose, insulin, TAG and NEFA did not alter significantly over the 30 min period following pyruvate infusion. The only significant metabolic changes observed were a 2.8-fold increase in lactate and a 3.5-fold increase in β-hydroxybutyrate at 30 min post pyruvate infusion (P <0.01; Figure 2).

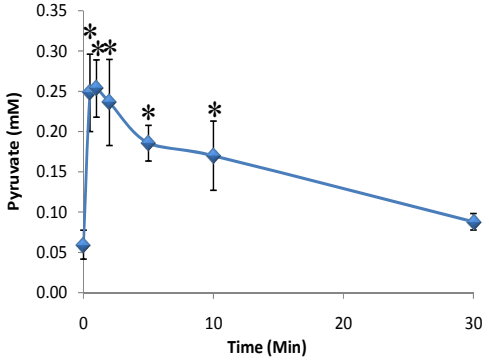

***Figure 1**. Plasma pyruvate concentration post infusion of 1 ml 80 mM sodium pyruvate (* P < 0.01)*

Discussion

This study was designed to investigate the metabolic effects of infusion of supra-physiological pyruvate concentrations during hyperpolarized MRS experiments. Our results show that plasma pyruvate concentration peaks 1 min post infusion. The maximum plasma concentration detected was ~250 µM. This is equivalent to concentrations reached during exercise or with certain dietary modifications thereby indicating that the pyruvate concentrations reached during hyperpolarized MRS experiments do not exceed levels occurring naturally within the body and therefore should not radically perturb systemic metabolism [3]. Consistent with this, no significant alterations in the concentration of glucose, insulin, TAG or NEFA were detected up to 30 min post pyruvate infusion. The significant increase in lactate concentration at 30 min suggests that excess pyruvate circulating in the plasma was converted to lactate via lactate dehydrogenase. The increase in β-hydroxybutyrate may indicate that the metabolism of pyruvate via pyruvate dehydrogenase results in a high concentration of acetyl CoA. Any excess acetyl CoA which cannot be immediately processed via the Krebs cycle could be converted to the ketone body β-hydroxybutyrate in the liver. One limitation of this study is that we were unable to differentiate metabolic differences caused by pyruvate infusion from those resulting from prolonged isoflurane anaesthesia which has previously been observed to affect metabolism when administered for prolonged periods [4]. However, because *in vivo* hyperpolarized MRS experiments are currently always conducted on anaesthetised animals, the metabolic changes observed in this study are representative of those occurring during hyperpolarized MRS experiments.

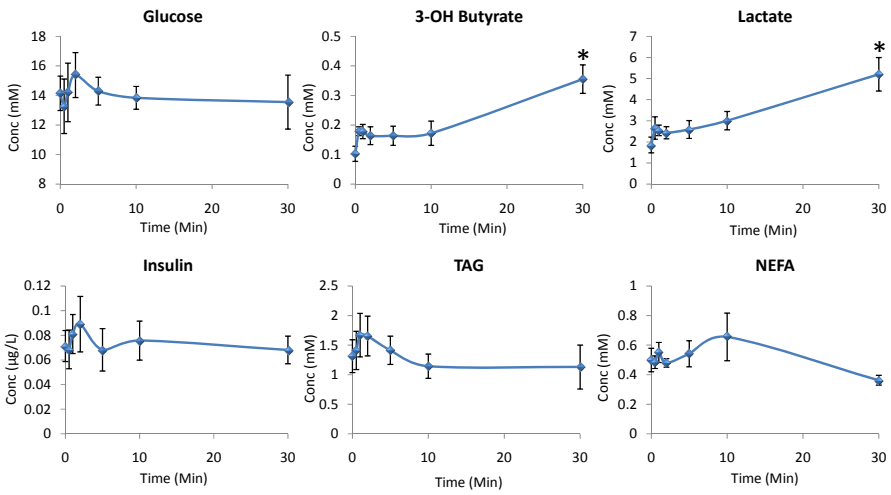

***Figure 2**. Plasma metabolic profiles post infusion of 1 ml 80 mM sodium pyruvate (* P < 0.01)*

References

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Acknowledgements

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