Hyperpolarised [2-^{13}C]Pyruvate Uniquely Reveals the Role of Acetylcarnitine as a Mitochondrial Substrate Buffer in the Heart

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Introduction In normal hearts, Krebs cycle flux is maintained by continuous citrate synthase-mediated condensation of acetyl-CoA with oxaloacetate. In turn, acetyl-CoA is produced from the oxidation of fatty acids, ketone bodies or glucose, via glycolysis and the enzyme pyruvate dehydrogenase (PDH). Hyperpolarised [2-^{13}C]pyruvate has been used in the isolated heart to observe the relationship between PDH flux and Krebs cycle metabolism in real time, using ^{13}C magnetic resonance spectroscopy (MRS) [1, 2]. Conversion of [2-^{13}C]pyruvate into the Krebs cycle related metabolites, citrate and glutamate, was observed. Further, a substantial fraction of the pyruvate oxidised by PDH was converted to acetylcarnitine [1]. Acetylcarnitine is produced by carnitine acetyltransferase (CAT), from mitochondrial acetyl-CoA and carnitine. This observation in the intact heart was consistent with that in cardiac mitochondria [3], which showed that a greater proportion of acetylcarnitine was produced from pyruvate-derived acetyl-CoA than from fatty acid-derived acetyl-CoA. These results point to clear involvement of CAT and the acetylcarnitine pool in cardiac substrate selection. However, the nature of the relationships that exists *in vivo* between acetylcarnitine, pyruvate oxidation, and Krebs cycle flux have yet to be determined. The aim of this study was to use hyperpolarised [2-^{13}C]pyruvate as a metabolic tracer to examine the role of acetylcarnitine in cardiac oxidative metabolism.

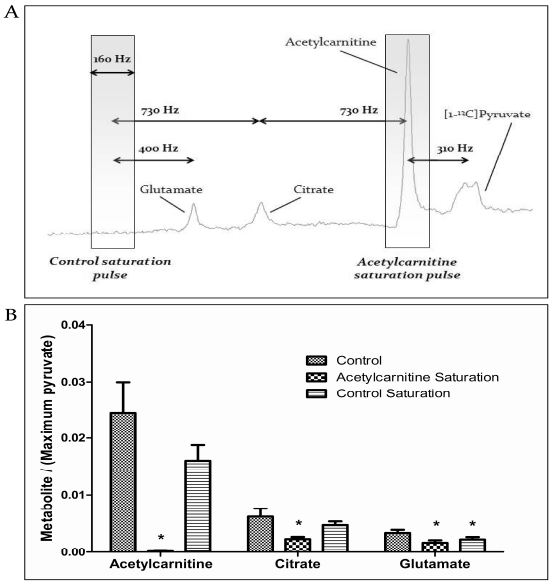

Figure 1 *Saturation transfer experimental design and results*

Methods

Perfused heart saturation transfer experiments: Five rat hearts were perfused in the Langendorff mode and placed in an 11.7 T vertical bore MR scanner. Hyperpolarized [2-^{13}C]pyruvate was infused into each heart a total of 3 times, and each of 3 acquisition protocols were implemented (Fig. 1A) (1) Control: acquisition with 1 s temporal resolution and a 30° flip angle [1]. (2) Acetylcarnitine saturation: a saturation pulse was applied to the acetylcarnitine resonance, which was 730 Hz downfield of citrate. (3) Control saturation: the saturation pulse was applied 730 Hz upfield of citrate to demonstrate that citrate was not affected by the RF saturation. A cascade of eight SNEEZE pulses [4] were applied to achieve saturation. Each SNEEZE pulse was 100 ms in duration and there was a 100 µs interval between pulses. No saturation was applied during signal acquisition (180 µs) and the effective full-width half-maximum bandwidth was 160 Hz when tested on acetone.

In vivo *experiments:* Male Wistar rats were examined with hyperpolarized [2-^{13}C]pyruvate in one of three metabolic states: (1) control, (2) following infusion of dichloroacetate (DCA), an activator of pyruvate dehydrogenase (PDH) or (3) following infusion of dobutamine, a β-adrenergic agonist that increases cardiac workload. Hyperpolarized [2-^{13}C]pyruvate was infused into the rats in a 7 T MR scanner and cardiac spectra were acquired with a surface coil every 1 s for 1 min. Conversion of pyruvate to [1-^{13}C]acetylcarnitine, [1-^{13}C]citrate and [5-^{13}C]glutamate was monitored.

Data analysis: All cardiac ^{13}C spectra were analysed using the AMARES algorithm in the jMRUI software package [5]. Quantified peak areas were normalised to maximum [2-^{13}C]pyruvate resonance area and the maximum resonance area of each metabolite was determined for each set of spectra. In acetylcarnitine saturation experiments, spectra were summed over the 50 s following pyruvate arrival at the perfused heart to yield a single spectrum with peak heights for [1-^{13}C]citrate, [5-^{13}C]glutamate, [1-^{13}C]acetylcarnitine and [1-^{13}C]pyruvate that were well above the noise. These single spectra were then quantified and used for subsequent analyses.

Results

Perfused heart saturation transfer experiments: The saturation pulse completely crushed the acetylcarnitine resonance and the control saturation experiments confirmed that the saturation pulse had a sufficiently low bandwidth to avoid direct RF effects on the citrate peak (Fig. 1B). The control saturation pulse did affect the glutamate peak, which was 300 Hz closer to the pulse than was citrate. Saturation of the acetylcarnitine resonance reduced the magnitude of the citrate peak by 63%. This indicated that, of all the [2-^{13}C]pyruvate-derived acetyl-CoA incorporated into the Krebs cycle over a 50 s period, the majority was initially converted into acetylcarnitine, prior to incorporation into the Krebs cycle. This effect was also observed as a 51% reduction in the glutamate resonance compared with controls.

In vivo *experiments:* In control rat hearts, the maximum [1-^{13}C]citrate and [5-^{13}C]glutamate resonances did not change in the presence of DCA or dobutamine (Fig 2). In control rat hearts, the maximum acetylcarnitine resonance was 0.023 ± 0.001. DCA infusion caused a 45% increase in the incorporation of ^{13}C into the acetylcarnitine pool. Dobutamine infusion caused a 31% decrease in the incorporation of ^{13}C into the acetylcarnitine pool. These observations indicate that mitochondrial acetylcarnitine buffers instantaneous changes in myocardial substrate supply and demand, both maintaining PDH flux by releasing CoASH in the case of excess acetyl-CoA supply and providing substrate that can be rapidly oxidised in the case of increased energy demand.

Discussion The high temporal resolution obtainable with hyperpolarised MRS has allowed us to detect close coupling between CAT and acetyl-CoA formed from carbohydrate. Further, our *in vivo* results revealed that the primary role of carbohydrate-derived acetyl-CoA may be to sustain the acetylcarnitine pool, in turn providing a rapidly responsive buffer of oxidative substrate within the mitochondria to complement the functions of phosphocreatine and longer-term substrate stores such as glycogen and triglycerides. The critical role of PDH in regulating the glucose-fatty acid cycle is undisputed; however, this study has demonstrated that high CAT activity and a dynamic acetylcarnitine pool are also fundamental in matching substrate supply with demand in the heart. Metabolism in aged and hypertrophic hearts is characterised by mismatches in glycolysis, glucose oxidation, and fatty acid oxidation, and also by decreased levels of myocardial carnitine (6,7). Consequently, use of hyperpolarised MRS to measure flux through the acetylcarnitine pool *in vivo* may provide an important clinical indicator of metabolic dysfunction in senescence and disease, and CAT activation via carnitine supplementation may represent a useful target to normalise metabolism in aged and diseased hearts (6,7).

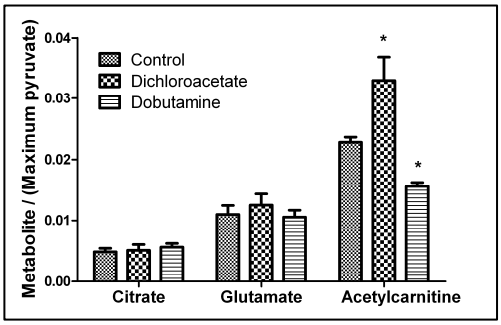

Figure 2 *Effect of DCA and dobutamine on [2-^{13}C]pyruvate metabolism*

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