Carnitine supplementation creates a cardiac reserve of free carnitine to enable buffering of excess acetyl units

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Introduction: Carnitine performs several vital roles in cellular metabolism. It facilitates the transport of fatty acids into the mitochondria and has recently been shown to buffer acetyl groups from excess acetyl-CoA, potentially recycling CoA for both β-oxidation and the TCA cycle[1]. Carnitine supplementation has been proposed as a treatment for conditions such as heart failure, where there is reported to be a decrease in free carnitine and a switch away from β-oxidation[2]. The idea of this supplementation is to drive fatty acid oxidation, by increasing the transport of fatty acids into the mitochondria. In this study, we used hyperpolarized ^{13}C magnetic resonance spectroscopy to detect alterations in cardiac metabolism following 2 weeks of carnitine supplementation in control animals. We also tested the buffering capacity of the free carnitine pool using dichloroacetate to stimulate increased pyruvate dehydrogenase activity, to understand if carnitine supplementation enable increased buffering of excess acetyl groups.

Methods: *Animals* – Five 275-330g Wistar rats received separate [1-^{13}C] and [2-^{13}C]pyruvate hyperpolarized scans at baseline as described below. The rats were provided with food and water *ad libitum* and scans were performed between 7 am and 1 pm during the fed state. Carnitine supplementation was started by the addition of 0.15% carnitine in the animals' drinking water for 2 weeks, levels of water consumption and the weight of the animals was monitored regularly. At the 2 week timepoint, animals again received separate [1-^{13}C] and [2-^{13}C]pyruvate hyperpolarized scans. A third scan using [2-^{13}C]pyruvate and dichloroacetate (DCA) was performed at the 2 week timepoint. DCA was injected into the animals 15 minutes prior to the hyperpolarized scan (30mg/kg, 1 ml bolus followed by a 0.5 ml infusion over 15 minutes). As a control for the DCA animals, another four 275-300g Wistar rats received DCA, followed by a [2-^{13}C]pyruvate scan.

Hyperpolarized ^{13}C MRS Protocol - [1-^{13}C] and [2-^{13}C]pyruvic acid were hyperpolarized, dissolved and neutralized as previously described[1,3]. An aliquot of 1 ml of 80 mM hyperpolarized pyruvate solution was injected over 10s via a tail vein catheter into an anaesthetised rat positioned in a 7T MR scanner. Spectra were acquired for 1 min following injection, using a slice-selective sequence localized to the heart (TR:1s, FA:15°, slice thickness:10mm). Signal was localised to the heart using a home-built ^{13}C RF surface coil. For [1-^{13}C]pyruvate scans, quantified peak areas were input into a kinetic model described by Atherton *et al*[4]. The rate of exchange of the ^{13}C label between pyruvate and its metabolites was termed ^{13}C label incorporation. ^{13}C label incorporation into the bicarbonate pool is a sensitive measure of PDH flux in the rat[4]. For [2-^{13}C]pyruvate scans, data was summed over the 60 seconds, to increase the available signal to noise. The data were then expressed as a ratio of pyruvate to metabolite, to account for polarization differences.

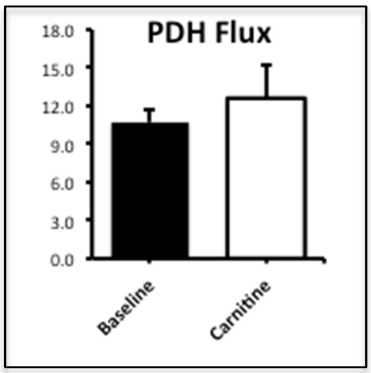

Figure 1 – PDH flux is unaltered after 2 weeks of carnitine feeding.

Results: Interestingly, 2 weeks of carnitine feeding did not alter PDH flux (Figure 1) or incorporation into acetylcarnitine (Figure 2). As previously reported[1], the infusion of dichloroacetate significantly increased the incorporation of label into the acetylcarnitine pool in control animals, due to an increase in PDH activity (not directly measured here). Interestingly, animals that were supplied with carnitine for 2 weeks, had a significantly greater increase in acetylcarnitine following DCA infusion, over that seen in the control DCA group (Figure 2).

Discussion: Short-term carnitine supplementation in control animals does not alter the metabolism of pyruvate under normal situations. However, during a time of increased acetyl group availability, such as DCA infusion, there is a carnitine reserve, which is able to buffer excess acetyl groups and allows the recycling of CoA back into β-oxidation or the TCA cycle. This holds clinical relevance as carnitine supplementation is expected to offer a level of protection in patients with heart failure[2]. This study demonstrates that the heart is able to provide a reserve of carnitine for use in times of excess acetyl-CoA.

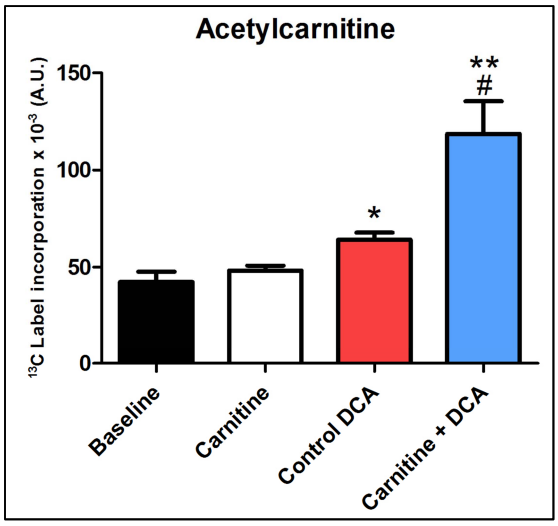

Figure 2 – [2-^{13}C]Pyruvate data shows a significant increase in label incorporation into acetylcarnitine following infusion of DCA. * p>0.05 and ** p>0.01 compared to Baseline and # p>0.05 compared to Control DCA.

References: [1]Schroeder, M *et al*, Circ Cardiovasc Imaging. 2012 Mar;5(2):201-9. [2]Lango, R *et al*, Cardiov Res, 2001. [3]Schroeder, M.A., *et al.* PNAS, 2008. **105**(33): p. 12051-6. [4]Atherton, H.J., *et al.* NMR Biomed. 2010 Aug 26.

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