Myocardial pyruvate dehydrogenase flux in long-chain acyl-CoA dehydrogenase knock-out mice

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Introduction: Patients affected by long-chain fatty acid β-oxidation (FAO) disorders may present with hypertrophic cardiomyopathy. It has been shown previously that in fed conditions, cardiac performance of long-chain acyl-CoA dehydrogenase knock-out (LCAD KO) mice is normal, but that in the fasted state, there are significant reductions in systolic and diastolic function [1]. It is hypothesized that glucose oxidation is unable to compensate sufficiently for the fatty acid oxidation defect to maintain myocardial energy homeostasis in fasted LCAD KO mice. The pyruvate dehydrogenase (PDH) complex plays an important role in the metabolic flexibility of the heart, as its activity determines the contribution of glucose oxidation to myocardial ATP production. In this study, we have used hyperpolarized ^{13}C-MRS of [1-^{13}C]pyruvate to investigate *in vivo* flux through PDH in wild-type (WT) and LCAD KO mice in both fed and fasted conditions to assess any differences in substrate utilization.

Methods: Male LCAD KO mice and C57BL/6 WT mice (n = 7 per genotype) were housed with ad libitum access to water and standard rodent diet. At ~20 weeks of age, hyperpolarized ^{13}C-MRS data were acquired as described below in the fed state and, on a separate day, after >20 hours of fasting.

Hyperpolarized ^{13}C-MRS - [1-^{13}C]pyruvate was hyperpolarized and dissolved as described previously [2]. Via a tail vein catheter, 0.2 mL of 80 mM hyperpolarized [1-^{13}C]pyruvate solution was injected into an anesthetized mouse positioned supine in a 7T MR scanner. ECG triggered ^{13}C MR spectra were acquired at 1 second temporal resolution for 1 minute following injection, using a 10° RF excitation pulse. Signal localization was achieved by carefully positioning a home built ^{13}C surface coil (Ø 20 mm) over the chest, with the heart in the sensitive area of the coil.

Data analysis - Spectra were analyzed using the time domain fitting software AMARES in jMRUI. Metabolite quantifications were input to a kinetic model [3], yielding the rate constant $k_{pyr\text{-}bicarb}$ [s^{-1}] of ^{13}C label incorporation from [1-^{13}C]pyruvate into the bicarbonate pool. This rate constant was used as a measure for PDH flux.

Results: Compared with WT controls, LCAD KO mice had similar myocardial PDH flux in the fed condition. The PDH flux in fasted LCAD KO mice was higher compared to fasted WT animals (P < 0.05, Figure 1). Interestingly, in fasted LCAD KO mice, examination of the spectra revealed peaks at chemical shifts corresponding to malate (182 ppm and 181 ppm) and aspartate (178 ppm and 175 ppm, Figure 2) [4].

Discussion: Myocardial PDH flux was normal in LCAD KO mice under fed conditions. However, after fasting the PDH flux decreased to a lesser extent in LCAD KO mice when compared to WT control animals. This suggests a compensatory role for glucose metabolism in the fasted LCAD KO mouse heart in an attempt to maintain myocardial energy homeostasis. In addition, the presence of ^{13}C-labeled malate and aspartate may point towards upregulated cardiac anaplerotic pathways in fasted LCAD KO mice. *Ex vivo* analysis of citric acid cycle intermediates and anaplerotic enzymes is ongoing to substantiate these *in vivo* findings.

References: [1] Bakermans, A.J., *et al*., 2011, *Circ Cardiov Imaging*, 4:558-65. [2] Golman, K., *et al*., 2006, *PNAS*, 103:11270-5. [3] Atherton, H.J., *et al*., 2010, *NMR Biomed*, 24:201-8. [4] Merritt, M.E., *et al*., 2009, *Proc ISMRM*, 17:532.

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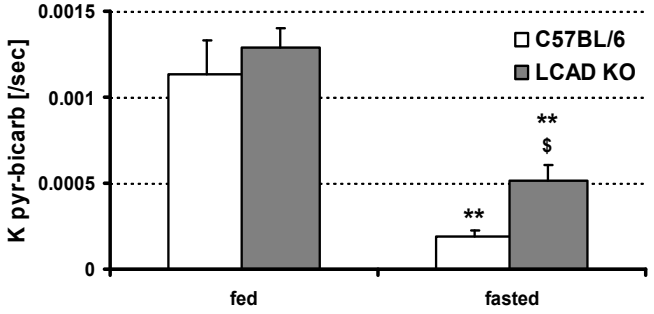

Figure 1 PDH flux was reduced in fasted WT and LCAD KO mice (**, P < 0.01 vs. fed). In fasted LCAD KO mice however, PDH flux remained much higher compared to fasted WT controls ($, P < 0.05).

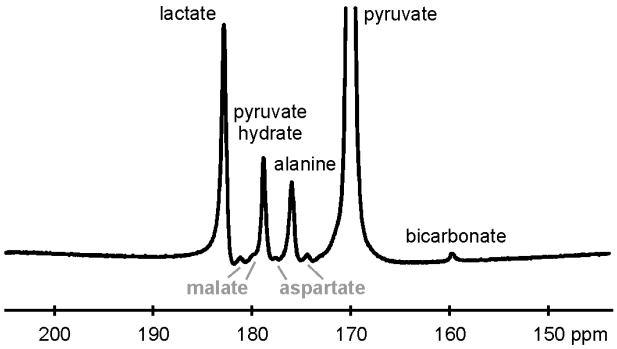

Figure 2 Summed spectrum of fasted LCAD KO myocardium, showing resonances assigned to malate and aspartate. Note the presence of bicarbonate, demonstrating the relatively high PDH flux after >20 hours of fasting.