Skeletal Muscle Metabolism Measured by Hyperpolarized 13C MR Spectroscopy

Jae Mo Mo Park[1], Sonal Josan[1], Dirk Mayer[2], Ralph Hurd[3], David Bendahan[4], Daniel Spielman[1], and Thomas Jue[5]

[1]*Radiology, Stanford University, Stanford, CA, United States,* [2]*Diagnostic Radiology and Nuclear Medicine, University of Maryland, Baltimore, MD, United States,* [3]*GE Healthcare, Menlo Park, CA, United States,* [4]*Centre de Resonance Magnetique Biologique et Medicale, Marseille, United States,* [5]*Biochemistry, UC Davis, Davis, CA, United States*

Target Audience

Researchers who are interested in muscle metabolism

Purpose

Conventional biochemistry often views lactate (Lac) as a hypoxemia indicator and a dead-end product of glycolysis, which muscle then exports to liver via the Cori cycle. Yet studies have detected Lac uptake in muscle and have correlated the turnover with O_2 consumption in resting and contracting muscle. The localized Lac flux/exchange in skeletal muscle suggests the presence of a Lac shuttle between muscle fibers and between the cytosol and the mitochondria. Moreover, it confers on Lac an unconventional role as a precursor[1]. In this study, we have followed hyperpolarized [1-^{13}C]Lac in rat leg muscle *in vivo* and have noted that muscle can indeed convert exogenous Lac to pyruvate (Pyr), alanine (Ala), and bicarbonate (Bic). The addition of dichloroacetate (DCA)[2], which activates Pyr dehydrogenase (PDH) by inhibiting Pyr dehydrogenase kinase (PDK), increases Bic but decreases Ala and Pyr. The addition of hyperpolarized [2-^{13}C]pyruvate (Pyr) in the presence of DCA produces peaks from acetyl-carnitine (ALCAR), acetoacetate (ACC) and glutamate (Glu), consistent with a PDH activation that enhances the TCA activity.

Methods

[1-^{13}C]Lac and [2-^{13}C]Pyr were hyperpolarized using a dynamic nuclear polarizer. Healthy male Sprague-Dawley rats were prepared for the study (n=11, 517-681g). Each animal was anesthetized and placed in a clinical 3T GE MR scanner, followed by an injection of the hyperpolarized Lac (n=7) or Pyr (n=3) solution through the tail vein. Metabolic kinetics was observed from the muscle using a ^{13}C surface coil (Ø=28mm, placed on top of right rectus femoris) and a dynamic free induction decay (FID) MR sequence (10^{o} hard pulse, temporal resolution=3s, spectral width/points=10kHz/4096, T_{acq}=4min). The acquired time-curves of spectrally resolved metabolites were fit to a modified 4-site exchange model[3] to calculate apparent conversion rate constants. Single time-point 3D images were acquired (n=1) for metabolite localization using a spiral chemical shift imaging (CSI) sequence (field of view=80x80x60mm^3, matrix=16x16x12, 3 spatial interleaves, 96 echoes, spectral bandwidth=972.8Hz, 20^{o} nominal flip-angle, T_{acq}=4s).

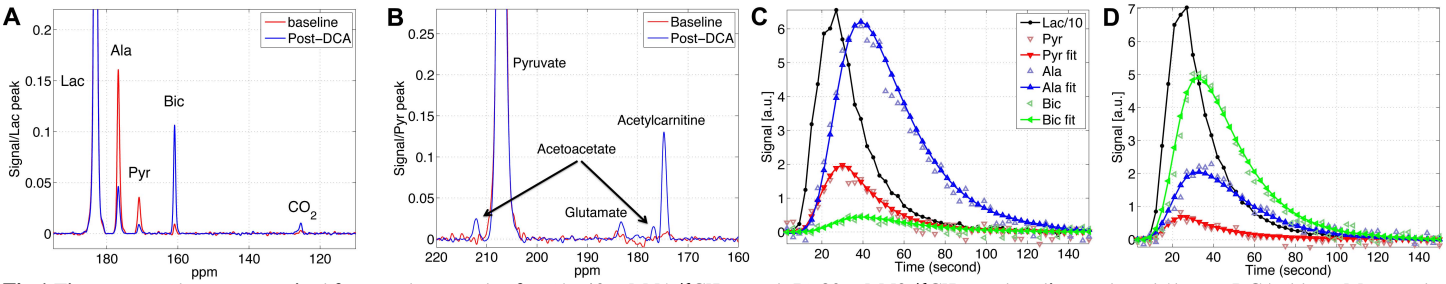

Fig.1 Time-averaged spectra acquired from rat leg muscle after (A) 40-mM [1-^{13}C]Lac and (B) 80-mM [2-^{13}C]Pyr at baseline (red) and 1h-post DCA (blue). Measured and fitted time-curves from [1-^{13}C]Lac at (C) baseline and (D) 1h-post DCA

Results and Discussion

Ala, Pyr, and Bic were detected from the injected [1-^{13}C]Lac. Metabolite ratios from the averaged (0-2min) spectra (Fig.1A) were in the control baseline vs. the post-DCA experiments (mean±se): Pyr/total carbon (tC): 0.034±0.002, 0.014±0.001, Ala/tC: 0.15±0.01, 0.073±0.009, Bic/tC: 0.011±0.002, 0.12±0.01, and Pyr/Ala: 0.23±0.02, 0.19±0.02. The apparent conversion rate constants were estimated from averaged time-curves (Fig.1C-D) as $k_{lac\rightarrow pyr}$=0.011±0.001s^{-1}, $k_{pyr\rightarrow ala}$=0.25±0.02s^{-1}, $k_{pyr\rightarrow bic}$=0.012±0.004s^{-1} at baseline and $k_{lac\rightarrow pyr}$=0.011±0.001s^{-1}, $k_{pyr\rightarrow ala}$=0.13±0.04s^{-1}, and $k_{pyr\rightarrow bic}$=0.16±0.06s^{-1} at 1h-post DCA. Imaging studies confirm that the metabolite signals originate primarily from leg muscle. In particular, Bic distributes fairly homogeneous over entire muscle area (Fig.2). Peripheral tissues and blood do not contribute significantly to Bic formation. When [2-^{13}C]Pyr was injected in the presence of DCA, mitochondrial metabolite peaks appear, corresponding to [5-^{13}C]Glu (Glu/tC = 0.013±0.002), [1-^{13}C]ACC (ACC/tC = 0.022±0.004), and [1-^{13}C]ALCAR (ALCAR/tC = 0.056±0.01) . Since the intrinsic Pyr pool size in muscle with or without DCA does not vary significantly (0.10 to 0.09 μmol/g)[4], the radically increased ^{13}C-Bic peak during DCA activation confirms PDH activation and a potential participation of Lac as a precursor in the TCA cycle to support oxidative phosphorylation. The [2-^{13}C]Pyr experiment results appear also consistent with an up-regulated PDH activity and an increased flow from Lac into the TCA cycle.

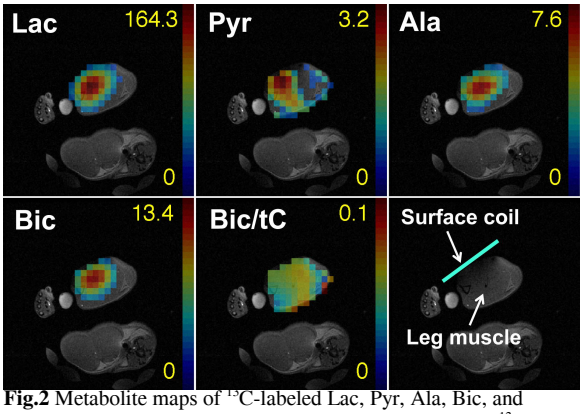

Fig.2 Metabolite maps of ^{13}C-labeled Lac, Pyr, Ala, Bic, and normalized Bic maps after a bolus injection of 40-mM [1-^{13}C]Lac. Images were acquired 1h-post DCA infusion using 3D spiral CSI

Conclusion

Muscle can rapidly convert Lac, consistent with its hypothesized role as an oxidative metabolism precursor.

References

1. Brooks GA et al, *J Physiol*. 2009; 587:5591-5600, **2.** Stackpoole PW et al, *J Clin Pharmacol.* 2003; 43(7):683-91, **3**. Park JM et al, *ISMRM*, 2013; 655, **4.** Schneider SH et al, *Metabolism.* 1981; 30:590-595.

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