Hyperpolarized 13C lactate as a substrate for in vivo metabolic studies in skeletal muscle

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Introduction:

Resting skeletal muscle has a preference for the oxidation of lipids compared to carbohydrates [1], with a shift towards carbohydrate oxidation during exercise. Lactate increases during exercise, and skeletal muscle is a major producer of lactic acid in the body. Skeletal muscle is also viewed as the most likely consumer of lactic acid as a respiratory fuel [2,3].The applicability of hyperpolarized [1-^{13}C]lactate as a precursor was demonstrated [4,5] and has several advantages for metabolic studies. It can be administered at physiological concentrations and does not interfere with other metabolic processes [6,7]. The aim of this study was to explore the use of hyperpolarized [1-^{13}C]lactate as a suitable probe for investigating carbohydrate oxidation in fed and fasted skeletal muscle in vivo.

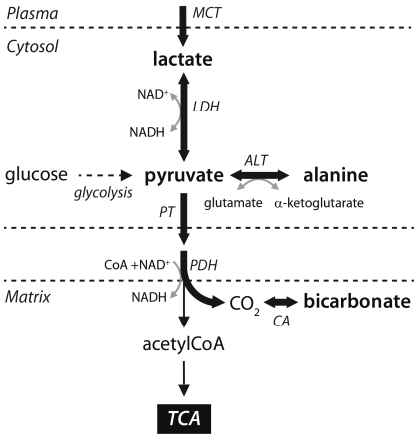

Fig 1. Lactate metabolic pathway

Materials and methods:

Sodium [1-13C]lactate was mixed with d8-glycerol in a 2:1 weight ratio to a concentration of 4.0 M with a final TEMPOL radical concentration of 50 mM. Frozen beads containing sodium [1-13C]lactate [4.0 M] mixed with d8-glycerol in a 2:1 weight ratio and a TEMPOL radical concentration of 50 mM were dynamically polarized in a 7 T polarizer for 90 min. The 13C polarization level at the time of the injection was 17 ± 2 %. Wild type male Sprague-Dawley rats were anesthetized, a catheter placed in the femoral vein for 1.5 mL of substrate delivery and in the artery for measurements of blood pressure, glucose and lactate concentrations, pH, PCO2, PO2 and bicarbonate concentrations. Fed animals (n = 5) and fasted animals (n = 5) were studied in a 9.4 T animal scanner using a custom-made surface coil, 30° adiabatic RF pulses applied every 3 s with ^{1}H decoupling. ^{13}C NMR time courses were summed and fitted to obtain relative metabolite ratios. Error bars indicate ± SEM.

Results and discussion:

Within seconds following the hyperpolarized [1-13C]lactate injection, lactate was transformed into pyruvate in the cytosol (Fig. 1) and the observation of bicarbonate indicates pyruvate transport into the mitochondrion for subsequent oxidation in the TCA cycle (Fig. 2a and 2b). Alanine attained higher signal intensities compared to pyruvate and this was ascribed to a larger endogenous alanine poolsize compared to that of pyruvate (Fig. 3a). No significant differences were observed in the time to maximum for both metabolites in both metabolic states. After a rapid elevation to 8.2 ± 1.5 mM following the injection, the arterial lactate concentration returned to baseline within 20 minutes. No physiological changes were observed as a result of the hyperpolarized lactate injection in terms of heart rate, blood pressure, pH, PCO2, PO2, bicarbonate and glucose levels. The resonance of 13C bicarbonate was absent in animals which were fasted overnight ($p<0.0005$), as a result of inhibited pyruvate dehydrogenase (PDH) flux (Fig. 3b). The ratio of pyruvate to lactate increased marginally ($p < 0.2$) and alanine decreased significantly from 0.072 ± 0.001 to 0.058 ± 0.001 ($p < 0.05$) (Fig. 3b). The alanine decrease could be ascribed to an alanine pool size change and, importantly, not due to a decrease in metabolic flux through alanine transaminase (ALT), a flux which is in fact expected to increase after an overnight fast. There was no relationship between the metabolite ratios and the administered lactate dose. Also no influence of repeated lactate injections in the same animal was observed.

Conclusion: Lactate is readily oxidized in resting skeletal muscle and significant changes observed in ^{13}C labeling of bicarbonate and alanine after an overnight fast were attributed to a decreased PDH flux and a decrease in cellular alanine concentration. The possibility of administering physiological doses of lactate makes it well suited to study carbohydrate metabolism. The measurement of alanine concentration changes and PDH flux offers the opportunity to probe skeletal muscle metabolic disorders.

Ref:[1]Brooks *et al.*(1994)[2]Gladden, (2004) [3] Brooks (2000) [4] Chen *et al.* (2008) [5] Mayer *et al.* (2012) [6] Yoshida *et al.* (2006) [7]Gladden *et al* (1994)

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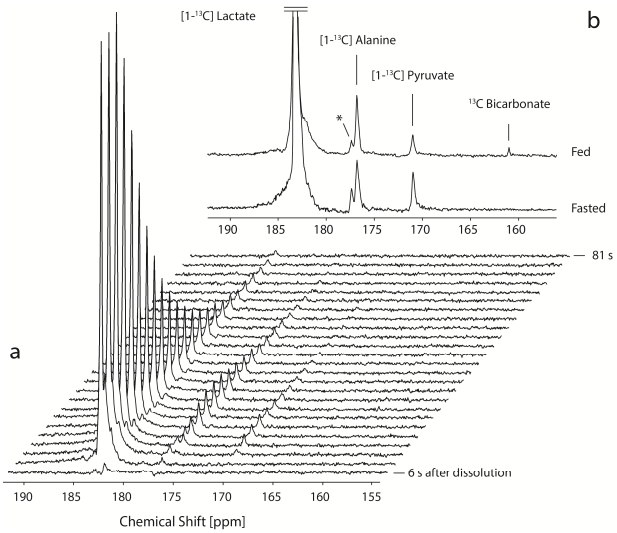

Fig. 2. (a) Metabolite time course and (b) summed spectra

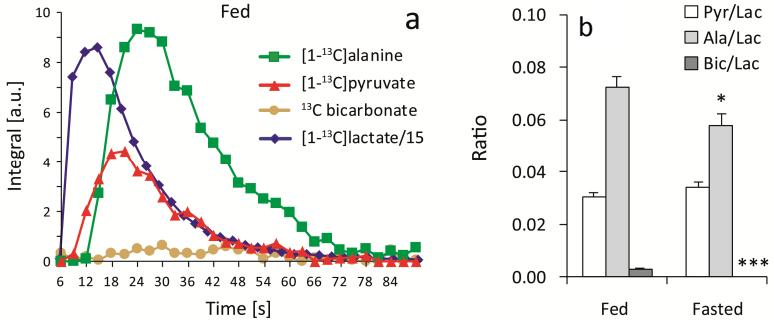

Fig 3. (a) Integrated metabolite time course (b) quantitative metabolite ratios.