Real-time Metabolic Probe into Non-Alcoholic Fatty Liver Disease with Hyperpolarized Carbon-13

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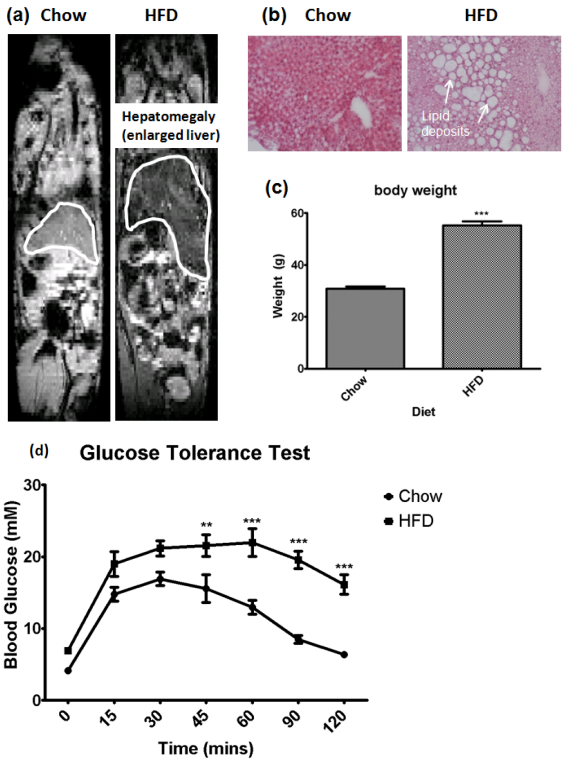
Introduction - Non-alcoholic fatty liver disease (NAFLD) is an increasingly prevalent condition that may progress to advanced stage liver disease. NAFLD encompasses a spectrum of hepatic disorders from steatosis to steatohepatitis, advanced fibrosis and cirrhosis. With increasing NAFLD occurrence in Asia, there is a need to better evaluate and manage this dysfunctional metabolic condition [1]. Currently, grading and staging of steatosis severity relies on invasive liver biopsy and biochemical serum tests [2]. High aminotransferase levels in the blood are indicative of the diseases but alternative objective diagnostic parameters are needed. The detection and transformation of hyperpolarized Carbon-13 biomolecules could provide such diagnosis in a non-invasive manner, and in addition provide real-time biochemical flux measurements in the liver. Here we demonstrate the measurement of pyruvate carboxylase activity as a potential biomarker of steatosis, by the detection of hyperpolarized [1-^{13}C] malate and [1-^{13}C]aspartate production.

Materials & Methods – Steatosis Mouse Model: C57Bl/6 mice were fed with normal chow (n=7) and high fat diet (HFD) (n=7) for 20 weeks. At 6 months of age, they were scanned in a preclinical 9.4 T MRI system for liver abnormality. Body weight was measured before hyperpolarized ^{13}C measurements. **Glucose Tolerance Test:** Mice were fasted overnight (16-18 h) with free access to water, upon which glucose was injected (2 mg/g of body weight). Blood samples (4 µl per time point) were collected from the tail vein at 0, 15, 30, 60, 90, and 120 min. Blood glucose concentrations were determined by using a glucometer. **Histology:** Liver morphology was assessed based on 5-µm oil red O–stained paraffin sections. **Pyruvate Polarization and Dissolution:** Approximately 40mg of [1-^{13}C] pyruvic acid, doped with 15mM trityl radical and 0.6mM of 3-Gd, was hyperpolarized in a polarizer. The sample was subsequently dissolved in a pressurized and heated alkaline solution, containing 100mg/liter EDTA, to yield a solution of 80mM hyperpolarized sodium [1-^{13}C] pyruvate with 30% polarization at physiological temperature and pH. Intravenous infusion of 300uL was followed by a 100uL saline flush. **Slice-selective 1D Spectroscopy:** 120 spectra of the metabolites were collected immediately upon intravenous infusion of hyperpolarized pyruvate (TR=1s, flip angle=20°, axial slice thickness=6mm, sweep width=40ppm, spectra points=2048). **Biochemical Flux Analysis:** MR spectra were analyzed with AMARES algorithm in the jMRUI package. Flux quantification was implemented according to [3]. To quantify metabolism, areas under the ^{13}C-lactate, ^{13}C-malate, ^{13}C-alanine, ^{13}C-aspartate, ^{13}C-bicarbonate, ^{13}C-pyruvate-hydrate and ^{13}C pyruvate peaks for initial 60 seconds were summed to yield the ratio Metabolite Carbon/total Carbon (total carbon-13 defined as the sum of all seven). Two-tailed Student t-test was implemented in comparative statistics (*, **, and *** indicate $p< 0.05$, 0.01, and 0.001 respectively).

Results and Discussion – After 20 weeks on HFD, mice were significantly heavier in body weight and developed hepatomegaly (Figure 1a,c). Subsequent histology reveals liver steatosis (Fig. 1b). From the Metabolite/total Carbon ratios of [1-13C]malate and [1-13C]aspartate (Fig. 2a), HFD mice exhibited significantly higher malate, aspartate and alanine pools. This could be attributed to the faster conversion of pyruvate to malate via pyruvate carboxylase/malate dehydrogenase (PC/MDH) and to aspartate via pyruvate carboxylase/aspartate aminotransferase (PC/AST) enzymes (Fig. 2b). The higher alanine aminotransferase (ALT) activity is an indicator of liver disease onset. Corresponding timecourses corroborated these results (Fig 2c,d). No significant changes were observed in lactate and bicarbonate production. It has been established that carbon anaplerosis via pyruvate carboxylase activity is enhanced in animals on a high fat diet, contributing to increased gluconeogenesis [4]. Therefore the higher measured malate and aspartate metabolite pools could be the result of an elevated PC flux, and possibly increased MDH and AST as well. As expected, the HFD mice were glucose intolerant.

Conclusion – We have demonstrated the use of hyperpolarized pyruvate to probe pyruvate carboxylase activity in-vivo. The PC flux, malate and aspartate pools could serve as alternative biomarkers of hepatic steatosis. Future work involves exploring their use in monitoring diabetes progression as well as aid in the prognosis of therapeutic intervention.



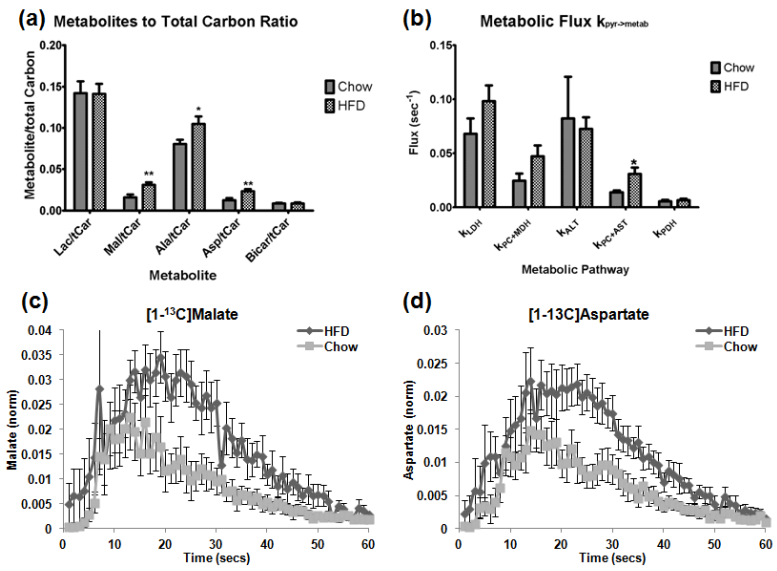


Figure 1: (a) T2-weighted MRI of animals on chow and HFD diets. (b) Histological comparison indicates fatty liver in HFD mice. (c) Elevated body weight as a result of 20 weeks of HFD. (d) Glucose tolerance test illustrates the progression to diabetes. Figure 2: (a) Ratio of metabolite pools to total carbon-13, consequent of hyperpolarized [1-^{13}C]pyruvate metabolism. (b) Corresponding fitted metabolic fluxes of pyruvate metabolism. (c, d) Timecourses of malate and aspartate production, upon intravenous infusion of hyperpolarized pyruvate.

References – [1] J Gastro Hepat 22; 778 (2007). [2] N Engl J Med 346(16); 1221 (2002). [3] J. Mag. Res. 202: 85 (2010); [4] Am J Physio Regul Integr Comp Physiol 281: R427 (2001).