In vivo enzymatic assay of carnitine acetyl transferase and acetylCoA synthetase using hyperpolarized acetate

Jessica A M Bastiaansen[1], Tian Cheng[1], Mor Mishkovsky[1,2], Arnaud Comment[3], and Rolf Gruetter[1,4]

[1]Laboratory of Functional and Metabolic Imaging, Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland, [2]Department of Radiology, Universite de Lausanne, Lausanne, Switzerland, [3]Institute of Physics of Biological Systems, Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland, [4]Department of Radiology, Universite de Lausanne and Geneva, Lausanne and Geneva, Switzerland

Introduction: The advent of hyperpolarized MR allows for study of real-time metabolism and offers the possibility to determine the kinetics of specific biochemical transformations *in vivo* [1]. It has been shown that the uptake and conversion of acetate, a main energy source in humans and rodents [2], can be probed in muscles after being hyperpolarized [3]. The enzymatic conversion of acetate to acetylcarnitine is dependent on the enzymatic activities of carnitine acetyl transferase (CAT) and acetylCoA synthetase (ACS), where carnitine plays an essential role in buffering acetylCoA [4,5]. Biochemical reaction rates and enzymatic activities reflect tissue health and are usually determined *in vitro*. The aim of the present study was to characterize the dynamics of hyperpolarized acetate metabolism in skeletal muscle *in vivo* and to determine the corresponding Michaelis-Menten kinetic parameters K_M and V_{max} of the transformation involving both CAT and ACS enzymes.

Materials and methods: [1-^{13}C]acetate samples (4.5M) solved in a 1:2 mixture of d_6-EtOD/D_2O containing 30mM TEMPO free radical were turned into frozen glassy beads and subsequently polarized in a custom-designed DNP polarizer for 2.5 h [6]. After dissolution, the samples were transferred within 2 s into a home-built infusion pump located inside the magnet bore and connected to a catheter inserted into a rat femoral vein. The 5 s long infusion of 1 mL hyperpolarized acetate solution was triggered 1 s later. The whole procedure was computer controlled in order to obtain an identical timing for all experiments. At the end of the *in vivo* acquisition, the residual solution that was left in the infusion pump was extracted and measured in a high-resolution 400 MHz spectrometer (Bruker) to determine the exact acetate concentration. Series of single pulse acquisitions were recorded using 30° adiabatic RF pulses applied every 3 s starting at the end of the infusion with a homebuilt surface coil. A two-compartment non-steady state metabolic model for one-site exchange was used to fit the hyperpolarized time courses and determine the kinetic rate constants *k* of the biochemical reaction and the initial reaction rates V_0. The model takes into account the effect of the 30° excitation pulses on the signals decay. The longitudinal relaxation time of [1-^{13}C]acetylcarnitine which was measured in blood at physiological temperature was fixed in the fitting procedure, leaving only 2 free parameters (*k* and the characteristic decay constant of acetate, which is a combination of T_1 and substrate inflow).

Results and discussion: The concentration of the 1 mL hyperpolarized [1-^{13}C]acetate bolus ranged from 30 mM to 200 mM and the time evolution of both substrate and product were measured *in vivo* in 17 different animals. Peak areas were measured by line fitting in jMRUI and a typical time course of the metabolic signals is displayed in figure 1. The *in vitro* T_1 of [1-^{13}C]acetylcarnitine in blood at T = 310 K was determined to be 14.9±0.1 s and used as a constant. Considering the concentration of the bolus, we assumed that the acetate pool can be approximated as fully labeled. Since the pool size of acetyl-CoA is substantially smaller than the acetylcarnitine pool [7], we assumed a unidirectional labeling of the acetylcarnitine pool during the short experimental time. As shown in Fig.1, the two-parameter model fits the time evolution quite accurately. Using this model, the kinetic rate constants were obtained as a function of hyperpolarized substrate concentration and this relation is displayed in Fig. 2, left panel. The tissue concentrations were obtained by dividing the amount of injected acetate by the animal volume. The determined kinetic rate constants represent both CAT and ACS activities. A fit to a Michaelis-Menten type model resulted in V_{max} = 3.4 ± 0.8 µM/s and K_M = 0.39 ± 0.19 mM (Fig. 2, right panel).

Conclusion: We conclude that the method described above can robustly characterize enzymatic activities *in vivo* in healthy skeletal muscle and that this method could be used to highlight pathological metabolism.

References: [1] J.H. Ardenkjaer-Larsen *et al.* , PNAS (2003) [2] E. N. Bergman, Physiol. Rev. (1990) [3] P. Jensen *et al.*, *JBC* (2010) [4] F. Stephens *et al*., J. Physiol. (2007) [5] A.L. Carter *et al.*, Febs Lett. (1981) [6] A. Comment *et al.*, Concept Magn. Reson. (2007). [7] L.L. Spriet *et al*., Am. J. Physiol. (1992)

Acknowledgements: This work was supported by the Swiss National Science Foundation (grants 131087, 124901 and 133562), the National Competence Center in Biomedical Imaging (NCCBI), the CIBM of the UNIL, UNIGE, HUG, CHUV, EPFL, and the Leenaards and Jeantet Foundations.

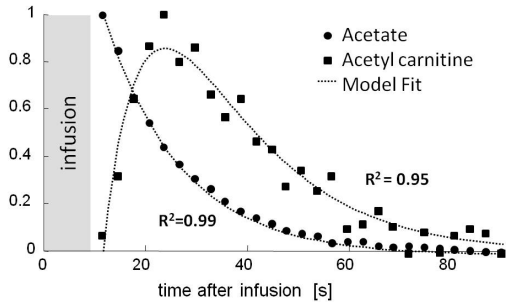

Fig. 1: Example of acetate and acetylcarnitine signal integrals vs. time. The data were fitted with the metabolic model to extract the kinetic rate constant.

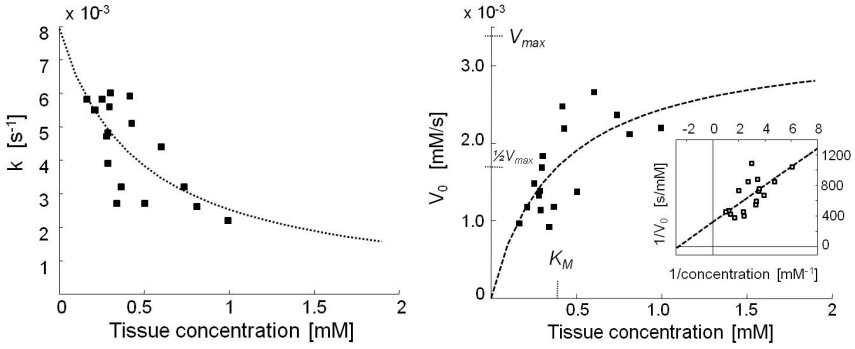

Fig. 2: Kinetic rate constants *k* (left) and initial reaction rates V_0 (right) as a function of the tissue concentration. The data were fitted to a Michaelis-Menten kinetic model with parameters K_M and V_{max}. The inset is a Lineweaver-Burk plot of the same data.