In vivo real time cardiac metabolism using hyperpolarized acetate

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INTRODUCTION: Hyperpolarized MRS allows for the study of fast biochemical processes associated with cardiac workload and gives the ability to differentiate between normal and pathological metabolism [1,2,3,4]. Acetate has been a successful tracer in assessing myocardial oxidative metabolism in PET and MRS and has been suggested as a marker of myocardial viability. After extraction from plasma, acetate is activated to acetylCoA which crosses the mitochondrial membrane through the carnitine shuttle complex after which it enters the TCA cycle as citrate via an irreversible condensation with oxaloacetate [5,6]. The formation of acetylCoA and acetylcarnitine, an important shuttle and buffer of acetyl groups, was previously detected *in vivo* after the infusion of hyperpolarized acetate [2], which is independent of pyruvate dehydrogenase (PDH) activity. Here we report the *in vivo* measurement of the metabolic conversion of acetate to acetylcarnitine and citrate, a TCA cycle intermediate, in cardiac muscle and perform a kinetic analysis of the observed dynamics.

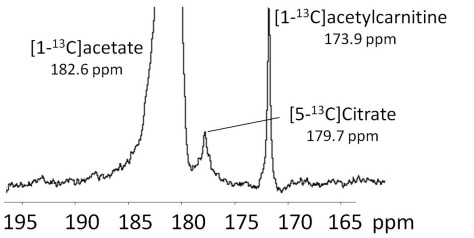

Fig. 1: Sum of 4 spectra (t = 21 s to 30 s). Acetate and two metabolic products (citrate and acetylcarnitine) were observed.

$$\frac{dM_{Ace}}{dt} = -R_{1,Ace}\left[M_{Ace} - M_{Ace,eq}\right] - kM_{Ace}$$

$$\frac{dM_{Acc}}{dt} = kM_{Ace} - kM_{Acc} - R_{1,Acc}\left[M_{Acc} - M_{Acc,eq}\right]$$

$$\frac{dM_{Cit}}{dt} = kM_{Acc} - R_{1,Cit}\left[M_{Cit} - M_{Cit,eq}\right]$$

Fig. 2: Three-compartment model equations

METHODS: All *in vivo* studies (n = 3) were performed in healthy Sprague-Dawley male rats on a 9.4T/31 cm MR scanner (Varian/Magnex) using home-built $^{13}C/^{1}H$ surface coils. Frozen glassy beads containing [1-^{13}C]acetate (4.5M) and TEMPO free radical (33mM) were dynamically polarized for 2 h [7], dissolved and transferred to an infusion pump within 2 s, and, 1 s later, 1 mL of the resulting hyperpolarized solution was automatically injected into a femoral vein within 5 s. MR acquisition started 3 s after the beginning of the infusion. The entire process including dissolution, transfer and MR acquisition was automated. Spectra were recorded using B_1-insensitive 30° adiabatic RF pulses to ensure the same excitation for the substrate and the observed metabolites and ^{1}H decoupling was applied during the acquisition. The measurements were performed with a repetition time of 3 s and triggered on the respiration and the cardiac cycle.

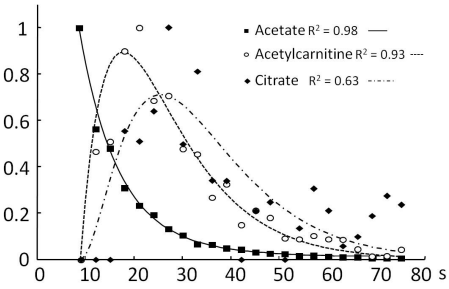

Fig. 3: Extracted peak areas from a typical experiment were normalized to their maximum and are displayed from the time of the injection. The extracted peak areas were fit to the three-compartment metabolic model (see Fig.2) to extract the kinetic parameters of the conversions.

RESULTS AND DISCUSSION: The metabolic time courses of hyperpolarized acetate (182.6 ppm) and its metabolic products citrate (179.7 ppm) and acetylcarnitine (173.9 ppm) were recorded in healthy myocardium *in vivo*. A spectrum is shown in Fig. 1. Using the FASTMAP shimming protocol, ^{1}H line widths of 20 to 30 Hz were obtained in a voxel size of 4x6x6 mm^3. The observed ^{13}C FWHM measured during the hyperpolarized MR experiments were between 30 and 40 Hz (localization achieved by placing the surface coil on top of the mouse heart). Peak areas were extracted using jMRUI and the time courses are displayed in Fig. 3. The maximum peak integral of acetate was observed 9 s after the beginning of the infusion, the maximum acetylcarnitine about 12 s later and the maximum citrate approximately 6 s after acetylcarnitine. This observation supports the role of carnitine as a shuttle for acetyl groups across the mitochondrial membrane. A three-compartment non steady-state metabolic model (Fig. 2) was used to fit the metabolic time courses. This model takes into account the effect of repeated excitations and contains 4 free parameters (R_{Ace}, $k_{Ace\text{-}Acc}$, $k_{Acc\text{-}Cit}$, R_{Cit}). The kinetic rate constant $k_{Acc\text{-}Cit}$ is representative for TCA cycle activity and thus myocardial oxidation. The T_1 relaxation time of [1-^{13}C]acetylcarnitine in blood was measured to be 14.9 s and used as a constant. The result of this fit is displayed in Fig. 3, with kinetic parameters $k_{Ace\text{-}Acc} = 1.5 \pm 0.2$ ms^{-1}, $k_{Acc\text{-}Cit} = 21 \pm 4.5$ ms^{-1}.

CONCLUSION: We conclude that by using hyperpolarized [1-^{13}C]acetate it is possible to study the *in vivo* real-time formation of acetylcarnitine and TCA cycle intermediates and thus directly measure energy metabolism in healthy myocardium. This method could be applied to study models of cardiac ischemia-reperfusion.

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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.