Abstract: #45310

Title: Kinetic analysis of acetylCoA synthetase activity in skeletal muscle

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Purpose / Introduction

Mathematical models are used for kinetic analysis in metabolic studies involving hyperpolarized substrates.Sufficient SNR is required to measure accurate time courses of metabolic products in order to fit the data with these models and determine specific kinetic parameters.To obtain a more robust differentiation between healthy and pathological metabolism spectra are often summed and the ratios of product/substrate used as disease markers[1].The real-time uptake and conversion of hyperpolarized acetate can be followed *in vivo*[1].The transformation of acetate into acetylcarnitine involves two enzymes,carnitine acetyltransferase (CAT) and acetylCoA synthetase (ACS),where carnitine plays an essential role in buffering excess acetylCoA[2,3].Here we show that the dynamics of the acetylcarnitine signal reflects uniquely the enzymatic activity ACS and that this activity can be extracted using spectral sums gaining sensitivity.

Subjects and Methods

Hyperpolarized [1-^{13}C]acetate was polarized and automatically infused into rats.Single pulse acquisitions were recorded using 30° adiabatic pulses applied every 3s with a homebuilt surface coil.A two-compartment non-steady state metabolic model for one-site exchange was used to fit the spectra and determine the kinetic rate constants k and initial reaction rates V_0.The longitudinal relaxation time of [1-^{13}C]acetylcarnitine ($1/R_{1,C}=T_1$=14.9±0.1s) was fixed in the fitting procedure,leaving 2 free parameters,k and the characteristic decay constant of acetate.Spectra were processed using Matlab and jMRUI.

Results

The acetate pool can be approximated as fully labeled.Since the pool size of acetylCoA is substantially smaller than the acetylcarnitine pool[5],we assumed a unidirectional labeling during the experimental time frame.We find that the dynamics of acetylcarnitine and the kinetic rate constants obtained from the model are reflecting that of ACS(fig.1).Time evolutions of substrate and product were measured *in vivo*.Fig.2A shows a linear relationship between the kinetic rate constants and the sum ratio,which has also been observed in cells using hyperpolarized pyruvate[6].Theoretically the ratio between the integral under the time evolution curve of substrate and metabolite gives the kinetic rate constant multiplied by T_1.This relationship was used to calculate the kinetic rate constants given the ratio of the summed spectra(fig.2B).The K_M and V_{max} were determined using metabolic modeling and the model free method,both providing similar results(fig.3).

Discussion/Conclusion

The kinetic rates derived from the observed metabolism of acetate reflects the enzymatic activity of ACS *in vivo* in skeletal muscle and this activity can be obtained without the use of complex metabolic models.

References

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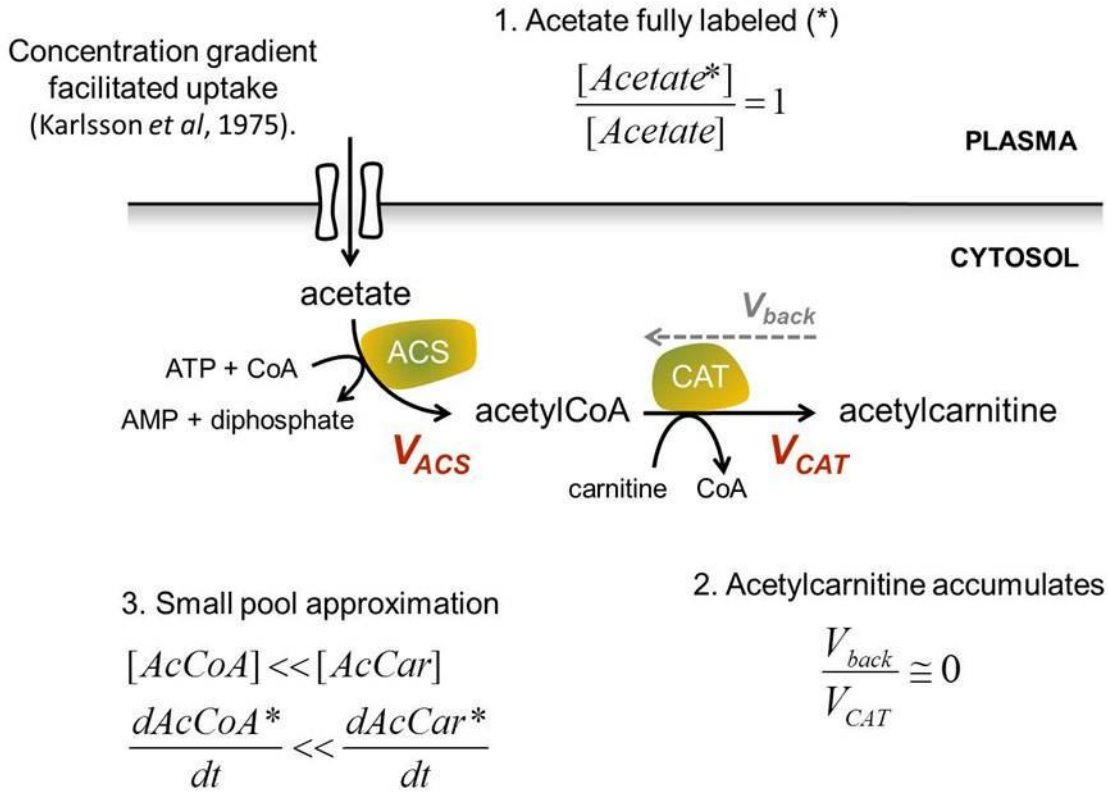

Figure 1. Scheme of hyperpolarized acetate metabolism. Acetate diffuses across cellular membranes and is convert do acetylCoA by acetylCoA synthetase (ACS). Acetyl carnitine transferase (CAT) converts acetylCoA to acetylcarnitine. In the analysis we assume that (1) the plasma acetate pool is fully labeled (^{13}C), (2) the flux from labeled acetylcarnitine to acetylCoA V_{back} is negligible and thus labeled acetylcarnitine accumulates and (3) we employ a small pool approximation since the acetylcarnitine pool size is much larger than that of acetylCoA.

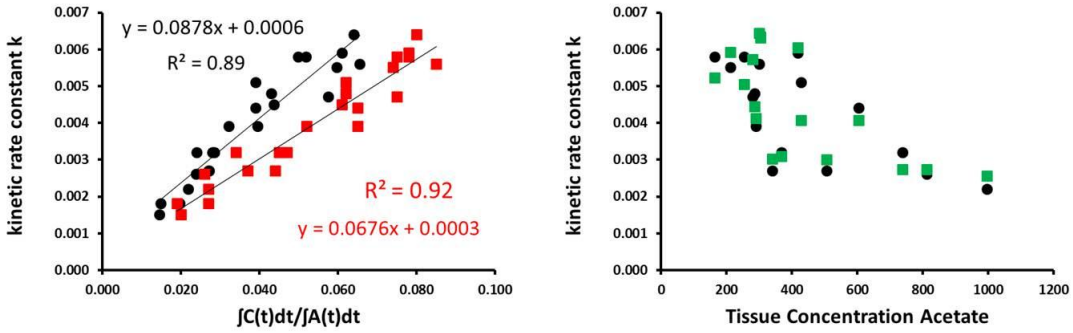

Figure 2A. The relationship between the kinetic rate constants *k* and the ratio of the sum of the hyperpolarized spectra (black spheres). C(t) denotes acetylcarnitine A(t) acetate. The individual spectral integrals were RF corrected before summing and are displayed as red squares. The line coefficient of the RF corrected data matches the longitudinal relaxation rate of acetylcarnitine as predicted.

Figure 2B. Relationship between the kinetic rate constants derived from the metabolic model (black) and the kinetic rate constants derived from the ratio of the spectral sum of acetate and acetylcarnitine (green).

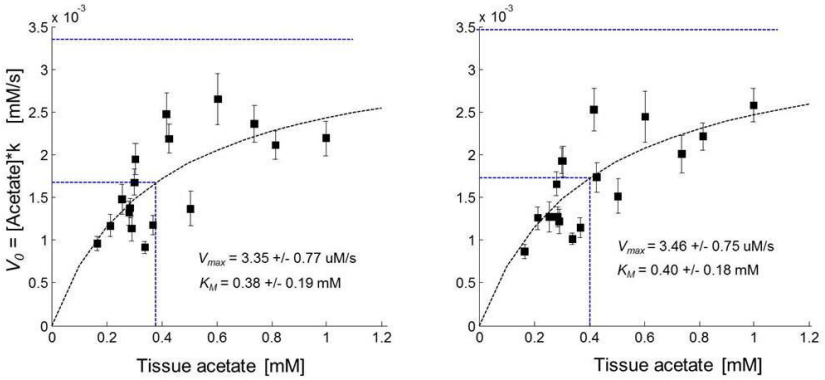

Figure 3. Michaelis Menten kinetics derived from kinetic rate constants obtained from the metabolic model (left panel) versus the ratios of the spectral sum (right panel). Error bars are in +/- standard deviation.