

# Flux in Metabolic Pathways from One $^{13}\text{C}$ NMR Spectrum

Johannes HGM van Beek  
Faculty of Biology, Vrije Universiteit  
De Boelelaan 1087  
1081HV Amsterdam, the Netherlands  
hvanbeek@bio.vu.nl

Harald GJ van Mil  
Theory of Complex Fluids Group  
Delft University of Technology  
the Netherlands  
h.g.j.vanmil@tnw.tudelft.nl

David JC Alders  
VU Medical Centre  
Amsterdam  
the Netherlands  
alders@physiol.med.vu.nl

## ABSTRACT

It used to be difficult to quantitate aerobic metabolic flux in mammalian tissue samples, especially in the multiple samples necessary to reveal spatial profiles of energy turnover [2]. We developed a pre-steady-state method to quantitate metabolic fluxes. The strategy is to infuse carbon-13 labeled substrates for aerobic metabolism, such as acetate and lactate. We obtain myocardial tissue samples after 5-7 min, before the isotopic steady state is reached. The multiplet structure in the NMR spectrum of tissue sample extracts is measured and analyzed with a model of the TCA cycle. Aerobic metabolism is relatively complex: the TCA cycle consists of three turns during which acetyl-CoA is metabolized. Carbon isotope leaves the cell in the form of carbon dioxide. Furthermore, TCA cycle intermediates are present at low concentrations which makes direct measurement by NMR spectroscopy impractical. However, the TCA cycle is in relatively fast exchange with the amino acid glutamate which is present at high concentration so that carbon-13 incorporation in glutamate is measurable by NMR spectroscopy. Thus the TCA cycle flux must be derived from label incorporation in a metabolite in a side branch.

Monte Carlo analysis of the computer model optimization strategy showed that the NMR spectrum of glutamate contains sufficient information to estimate several metabolic parameters simultaneously. Six metabolic parameters can be quantitated from just nine measurable carbon-13 multiplets of glutamate. These six parameters are the TCA cycle flux, the transport time from label infusion to incorporation in acetyl-CoA, the fraction of acetyl-CoA derived from the labeled substrate, the size of the glutamate pool that is reachable by label, the anaplerotic flux (consisting of other metabolites besides acetyl-CoA entering the TCA cycle) and the transamination rate which exchanges carbon skeletons between the TCA cycle and amino acid pools.

The method has been validated against a gold standard for measurement of aerobic metabolism [3]. Thus metabolic flux can be quantitated based on one high-resolution NMR spectrum, measured at one point in time, in contrast to previous methods

which used multiple time points measured on large tissue regions with low resolution NMR [1,4,5].

The carbon-13 measurements reveal major heterogeneity of energy turnover in cardiac tissue, despite the heart's apparent structural homogeneity. We conclude that the analysis of pre-steady-state carbon-13 NMR spectra based on computer models constitutes a practical method to measure aerobic metabolic flux in biological samples.

## REFERENCES

- [1] Chatham, J.C., Forder, J.R., Glickson, J.D. and Chance, E.M. Calculation of absolute metabolic flux and the elucidation of pathways of glutamate labeling in perfused rat heart by  $^{13}\text{C}$  NMR spectroscopy and nonlinear least squares analysis. *J. Biol. Chem.* 270 (1995), 7999-8008.
- [2] Van Beek, J.H.G.M., Van Mil, H.G.J., Alders, D.J.C., Groeneveld, A.B.J., Van Lambalgen, A.A., De Kanter, F.J.J., Harrison, G.J. and Bussemaker, J. Heterogeneity of local metabolism and perfusion. *Adv. Exp. Med. Biol.* 471 (1999), 271-281.
- [3] Van Beek, J.H.G.M., Van Mil, H.G.J., De Kanter, F.J.J., King, R.B., Alders, D.J.C. and Bussemaker, J. A  $^{13}\text{C}$ -NMR double labeling method to quantitate local myocardial oxygen consumption using frozen tissue samples. *Am.J.Physiol.* 277 (1999), H1630-H1640.
- [4] Weiss, R.G., Stern, M.D., De Albuquerque, C.P., Vandegaer, K., Chacko, V.P. and Gerstenblith, G. Consequences of altered aspartate aminotransferase activity on  $^{13}\text{C}$ -glutamate labelling by the tricarboxylic acid cycle in intact rat hearts. *Biochim. Biophys. Acta.* 1243 (1995), 543-548.
- [5] Yu, X., Alpert, N.M. and Lewandowski, E.D. Modeling enrichment kinetics from dynamic  $^{13}\text{C}$  NMR spectra: theoretical analysis and practical considerations. *Am. J. Physiol.* 272 (1997), C2037-C2048