# Modeling of $^{13}$C MRS data of cerebral glucose metabolism comparing mild hypoglycemia with euglycemia in humans

**K. C. van de Ven[1], M. van der Graaf[1,2], A. A. Shestov[3], B. E. de Galan[4], C. J. Tack[4], P-G. Henry[3], and A. Heerschap[1]**

[1]Radiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [2]Clinical Physics Laboratory, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [3]Center for Magnetic Resonance Research, University of Minneapolis, Minneapolis, Minnesota, United States, [4]General Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands

## Introduction

During hypoglycemia the supply of the brain with its primary energy substrate, glucose, is reduced. The metabolic effects of this condition have been examined by $^{1}$H MRS under hypoglycemia [1] or after several hypoglycemic periods [2]. Studies under hypoglycemia have been done by $^{13}$C MRS as well, but with alternative substrates like acetate [3] and lactate [4]. Thus the direct effect of hypoglycemia on cerebral glucose metabolism remains unclear.

*Aim:* to study the direct effects of hypoglycemia on cerebral glucose metabolism by $^{13}$C MRS with the infusion of [1-$^{13}$C]glucose. This involved: a) the registration of conversion of $^{13}$C-label from glucose into isotopic positions in downstream amino acids (e.g. glutamate-C4 and C3) and b) modeling of this data by a simple one-compartment model [5] to estimate metabolic kinetics in the brain. The model included $^{13}$C labeling of plasma lactate and exchange with $^{13}$C labeled brain lactate.

## Materials and methods

Eight healthy volunteers (4 male/4 female, 23.2±2.5 yrs old) were subjected to two hyperinsulinemic (60 mU·m$^{-2}$·min$^{-1}$) glucose clamps after an overnight fast [6]. They were clamped at euglycemia (~5mmol/L) on one day and at hypoglycemia (~3mmol/L) on another day, 4 weeks apart. The clamps were designed to have stable and comparable levels of [1-$^{13}$C]glucose in plasma. They started with a bolus of 6 g of 100% [1-$^{13}$C]glucose 20 % (w/w) solution infused over 10 minutes to increase plasma glucose $^{13}$C enrichment followed by two hours by infusion of respectively 40% and 50% enriched [1-$^{13}$C]glucose at euglycemia and hypoglycemia at a variable rate to maintain target plasma glucose levels. Arterial blood was sampled in 5 min intervals to determine plasma glucose concentration and [1-$^{13}$C]glucose isotopic enrichment by high resolution $^{1}$H NMR. For in vivo measurements a DEPT sequence was used in combination with ISIS localization and $^{1}$H decoupling. $^{13}$C-MRS acquisition (72 scans, TR=2s, duration=2.5 min) of a voxel of ~125 ml in the occipital brain tissue was started 20 min before clamping to obtain 8 reference spectra, and continued throughout the entire clamp (± 2 h). All experiments were performed at 3T with an optimized volume coil for $^{1}$H with a CP surface coil insert for $^{13}$C [7]. The FIDs of 8 reference spectra were averaged and subtracted from all FIDs to remove natural abundance signals and baseline distortions due to residual lipid signals. To enhance SNR the FIDs were added in running averages of 15 min (6 spectra). These spectra were fitted in jMRUI with the AMARES algorithm. For quantification the natural abundance $^{13}$C Myo-inositol signal was assumed to reflect a tissue level of 6 µmol/g. In addition, the data were corrected for the acquisition sequence pulse profiles (measured in a phantom).

Modeling was performed with a standard one-compartment model as previously used in [5], where plasma glucose and enrichment of plasma glucose are used as input functions (Fig. 1). Glucose uptake into the cell is modeled by Michaelis-Menten kinetics [8], and additionally in this model [3-$^{13}$C]lactate in plasma is estimated to increase linear over time to 10% after two hours, based on analysis of HR NMR data at the end of the experiment of a subset of subjects. Assuming a lactate plasma concentration of 1 µmol/g, this results in a concentration of 0.1 µmol/g [3-$^{13}$C]lactate in plasma after two hours. Further assumptions for the modeling were [Glutamate]$_{brain}$ =10 µmol/g, [Glutamine]$_{brain}$ =4 µmol/g.

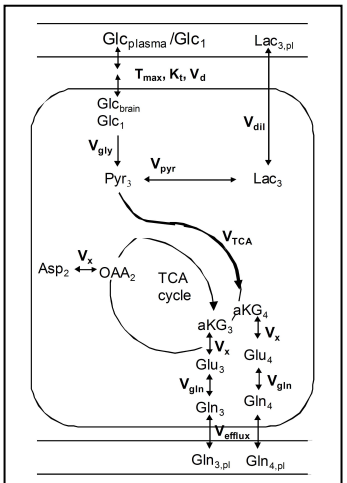


**Figure 1: Graphical description of the one compartment metabolic model**

## Results

In glutamate $^{13}$C label appears first at the C4 and then at the C3 position both under euglycemia and hypoglycemia (see Fig 2). During the first 20 min of the experiments the clamp procedure was similar for both glucose conditions, resulting in comparable glutamate curves. Later in the experiment the [1-$^{13}$C] enrichment in plasma glucose starts to deviate between groups, causing lower percentage enrichment during hypoglycemia (30%) versus euglycemia (35%) even though a higher glucose enrichment was used for infusion during the hypoglycemic experiment to compensate for less total glucose infusion under this condition.

The experimental curves are well fitted by the one-compartment model, when assuming plasma lactate C3 being labeled by 5% per hour (see Fig 2, solid and dotted lines for eu- and hypo euglycemia respectivily). The corresponding flux values (in µmol/min/g) through the Tricarboxylic acid (TCA) cycle for the euglycemic and hypoglycemic experiment respectively are $V_{TCA}$=0.58 and 0.54.

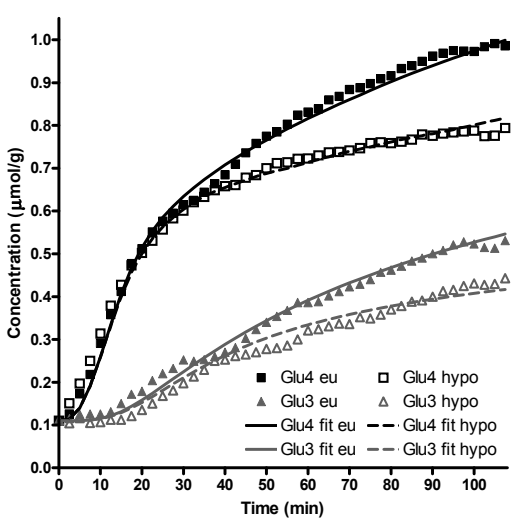


**Figure 2: Mean glutamate C4 (squares) and C3 (triangles) $^{13}$C uptake curves of all volunteers together with their best fits (solid/dotted lines) during euglycemia (closed symbols/solid lines) and hypoglycemia (open symbols/dotted lines) assuming a plasma lactate enrichment of 5% per hour.**

## Discussion and conclusion

Volunteers in this study suffered from clear hypoglycemic symptoms. Nevertheless, under the assumption of identical labeling patterns of [3-$^{13}$C]lactate in plasma, $V_{TCA}$ values are comparable for eu- and hypoglycemia. This indicates that normal glucose metabolism in the brain is maintained and hypoglycemia as applied in this study does not affect major brain functions.

There are several explanations for this outcome; first of all the induced hypoglycemia is mild, it can be that even though the rest of the body is alarmed by a glucose deficiency, this is not a rate limiting concentration for brain metabolism. Furthermore, these volunteers are aware of the hypoglycemia, this awareness possibly increases brain activity in the occipital region, and therefore $V_{TCA}$ is maintained during hypoglycemia in this brain region [9]. These results are also in agreement with a study on rats [10], where direct hypoglycemia did not influence cerebral glucose metabolism in control animals.

**References: 1.** Bisschof MG, et al., Eur J Clin Inv 2006; **2.** Criego AB, et al., J Neurosci Res 2005; **3.** de Feijter H, et al., proc ISMRM 2009 **4.** Mason GF, et al., Diab 2006; **5.** Henry PG, et al., J Neurochem 2002; **6.** van de Ven, et al., J Neursci Meth, in press; **7.** Klomp DW, et al., MRM, 2006; **8.** Gruetter, et al., AJP, 2001; **9.** Bingham, et al., Diab 2005. **10.** Jiang, et al., Diab 2009.

**Acknowledgments:** Dutch Diabetes Research Foundation (Grant 2004.00.012) and NIH (Grant R21 DK069881) for financial support.