Simplified ^{13}C metabolic modeling for simplified measurements of cerebral TCA cycle rate *in vivo*

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^{13}C NMR spectroscopy combined with metabolic modeling is a unique tool to measure TCA cycle rate *in vivo* [1,2]. The measurement relies on the i.v. infusion of a ^{13}C-enriched substrate, typically glucose labeled at C1 and/or C6, and in the dynamic detection by NMR spectroscopy of the progressive incorporation of ^{13}C at positions C3 and C4 of glutamate. These measured enrichments are related to the TCA cycle flux (V_{TCA}) using a metabolic model, which is a set of differential equations describing the temporal evolution of ^{13}C enrichments as a function of the infused substrate and of metabolic parameters such as fluxes and transporters kinetics. In particular, the passage of glucose through the blood-brain barrier and the amount of ^{13}C-glucose available inside the brain is calculated based on (i) assumed values of the kinetics constants; and (ii) the glucose and ^{13}C-glucose plasma concentrations time-courses, which impose blood sampling throughout the experiment followed by glucose concentration measurement and ^{13}C enrichment determination. However, the knowledge of Michaelis-Menten parameters is not straightforward, since they might vary between species, tissues and pathological conditions such as diabetes [3]. In addition, the experimental burden associated to glycemia measurements complicates significantly the already complex ^{13}C experiment, especially for small animal like mice. In the present work, it is demonstrated that, under experimental conditions, the ^{13}C fractional enrichment (FE) of the brain pyruvate/lactate pool is extremely stable. In consequence, a simplified and robust metabolic model is derived which describes ^{13}C incorporation starting directly from lactate, avoiding any assumptions about the glucose transport or any blood glucose time-course measurements.

Methods

Acquisition of blood glucose time-courses: Experiments were performed on a Bruker 3T system. Two macaque monkeys were fasted overnight and anesthetized by continuous i.v. propofol infusion. Glucose infusion was performed according to a protocol that has been extensively validated and used in our laboratory [4-6]. It started with a 3-min bolus of 99% enriched uniformly labeled glucose ([U-$^{13}C_6$]glucose) to reach a three-fold increased glycemia. Then a 2:1 mixture of [U-$^{13}C_6$] and non-labeled glucose was infused continuously at a lower rate, yielding a very stable blood glucose FE until the end of the infusion (2h). Glutamate C3 and C4 time-courses were measured by NMR spectroscopy with a 9-minute temporal resolution, as described elsewhere [4]. During the first 15 min of infusion, glycemia was measured every 5 min with the use of a Onetouch glucose meter (Lifescan Inc., Milpitas, CA). Glycemia was then measured every ~20 min until the end of the protocol. The infusion rate was adjusted so that the blood glucose concentration remained 2 to 3 times greater than the basal glycemia. In parallel, we collected blood samples in order to measure glucose FE using high-resolution NMR spectroscopy [4].

Numerical simulations: Numerical simulations were performed based on a single compartment metabolic model (fig. 1) implemented in Matlab (The MathWorks Inc., Natick, MA), including V_{TCA}, the glycolysis flux V_{gly} and the exchange rate between oxoglutarate and glutamate V_X. In its complete version, the model accounts for glucose transport through the blood–brain barrier according to reversible Michaelis-Menten kinetics (T_{max}, K_t and V_d). An influx of unlabeled lactate from the blood to the brain at rate V_{in} is taken into account, as well as an lactate efflux from the brain to the blood (V_{out}), which allows accounting for label dilution. In its simplified version, the model excludes all pathways before the Pyr/Lac pool.

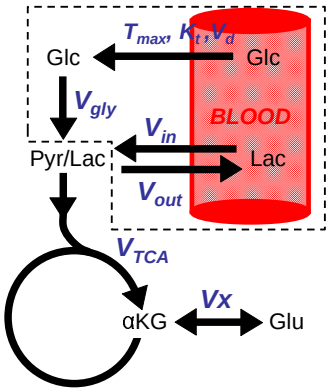

Fig. 1: The metabolic model used. In the simplified version, pathways within the dashed box are ignored.

Results

Stability and reproducibility of lactate FE time-course: First, numerical simulations show that the time-course of lactate FE in the brain does not depend much on experimentally measured blood glucose. Fig. 2A displays the time-courses for blood glucose concentration and FE, as measured for one of the monkeys. On fig. 2B, the corresponding glucose and lactate FE in the brain are plotted (simulated with V_{gly}=0.5, V_{in}=V_{out}=0.3, T_{max}=5, K_t=5, V_d=0.7), showing that despite the relatively unstable glucose time-courses in fig. 2A, FE are extremely stable and reproducible, showing a ~linear increase during the first 10 minutes (R^2=0.96 and 0.98 for the 2 monkeys), then stabilizing around a plateau value (s.d.<3% for t>10min). Second, when varying significantly T_{max}, K_t and V_d as well as V_{gly}, V_{in} and V_{out}, similar simulations show that lactate FE is again extremely stable, following the same 10min linear increase followed by a plateau. The only notable effect of changing these parameters is actually changing the value at the plateau.

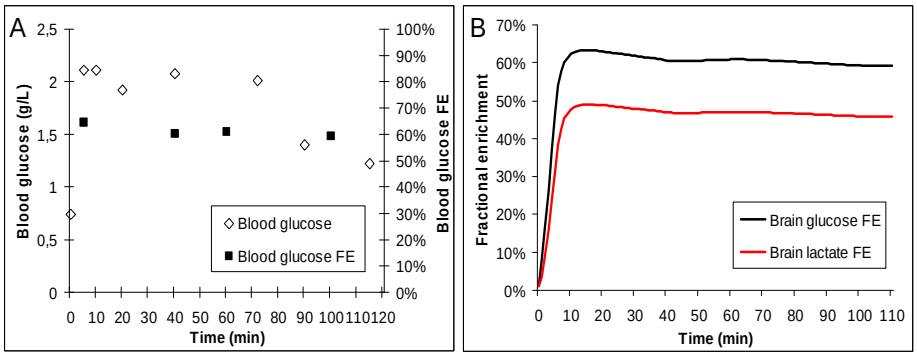

Fig. 2: A) Blood glucose and ^{13}C-glucose FE, as measured for one of the monkey. B) The corresponding simulated glucose and lactate FE in the brain appear very stable.

	Complete model	Simplified model
Mac 1	0.59±0.03	0.58±0.03
Mac 2	0.58±0.04	0.56±0.03

Table 1: V_{TCA} values (in µmol.g^{-1}.min^{-1}) derived using the complete model and the reduced model for both monkeys.

Accuracy and robustness of the simplified model: Lactate FE determines the entry of label in the TCA cycle. Taking advantage of the stability of lactate FE time-course, a simplified model can be derived by removing all pathways before the lactate pool (dashed box in fig.1). Instead the lactate FE time-course is modelled by a linear increase for t<10min and a stable plateau for t>10min, the value of the plateau being fitted from the enrichment time-courses of glutamate C3 and C4. This is in essence equivalent to fitting label dilution, as it is achieved in the complete model by fitting V_{in} and V_{out}.

V_{TCA} values derived from experimental ^{13}C-glutamate data using either the simplified or the complete model appear to be identical, taking standard deviation into account (s.d. was evaluated by Monte-Carlo simulation). Results are summarized in table 1. This proves the ability of the simplified model to yield the same results as the validated, complete model.

Conclusion

This work proves that, when using a ^{13}C-glucose infusion protocol designed to yield a stable glucose FE in the blood, lactate FE rapidly reaches a stable plateau in the brain. This allows the derivation of a simplified model which does not require any assumption about Michaelis-Menten parameters, which are susceptible to vary between different conditions. In addition, the simplification of the model may have an impact on the way experiments are performed and on their costs, since it avoids the burden of blood sampling and analysis.

[1] De Graaf *et al.*, NMR Biomed (2003); [2] Henry *et al.*, MRI (2006); [3] Cornford *et al.*, Neurochem Res (1995); [4] Boumezbeur *et al.*, MRM (2004); [5] Boumezbeur *et al.*, JCBFM (2005); [6] Valette *et al.*, MRM (2008)