Cerebral energy metabolism and neurotransmission in a schizophrenia mouse model: a combined ^{1}H-[^{13}C] MRS and ^{13}C MRS study at 14.1T

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Synopsis

In this study, we applied interleaved localized ^{1}H-[^{13}C]-MRS and direct ^{13}C MRS with polarization transfer to characterize brain energy metabolism in the early development of a GCLM-KO mouse model with infusion of [2-^{13}C]acetate. This strategy enabled to measure simultaneously the [2-^{13}C]acetate input function, glutamate and glutamine C4 and C3 enrichment and pool size changes. Two-compartment metabolic modelling was then applied to characterize mitochondrial metabolism and glutamate/glutamine cycling and compare it to a control group.

Introduction

Schizophrenia(SZ) is a neurodevelopment disorders involving both genetic and environmental risk factors. Large amount of ^{1}H MRS studies have shown changes in glutamate and glutamine levels in brains of schizophrenic patients are generally interpreted as markers of glutamatergic dysfunction[1]. How this relates to alterations of the glutamate/glutamine neurotransmission cycle remains unclear. Mice with genetic deletion (knockout) of the GCLM gene (KO) display a chronic GSH deficit and phenotypic anomalies that are similar to those observed in patients[2]. These deficits are aggravated by additional oxidative stress during particular developmental periods. To investigate this, we investigated brain energy metabolism and glutamate-glutamine cycling with dynamic ^{13}C MRS under infusion of [2-^{13}C]acetate in GCLM-KO mice under early postnatal oxidative challenge.

Methods

Animal preparation

Two groups of 30 days old C57BL6/J mice (9.3-17.0g) were used in this study: a first group of GCLM-KO mice (n=13, 7 females) with early postnatal oxidative challenge by administration of dopamine uptake inhibitor GBR12909 during postnatal development (P10-20), and a second group of wild type (WT) mice (n=10, 6 females) as control (Figure 1A). Animals were anesthetized by a mixture of air and O_2 (1:1 ratio) and 0.9-1.2% isoflurane and prepared for femoral vein substrate infusion. During the whole experiment, the body temperature was kept at 37±0.5°C by a tubing with circulating warm water. The respiration rate and the body temperature were monitored by a small animal monitor (SA Instruments Inc.). All animal procedures were performed according to federal guidelines and were approved by the local ethics committee.

Dynamic ^{13}C MRS protocol

[2-^{13}C]acetate was chosen as labeling substrate to be more sensitive to astrocytic metabolism and glutamate/glutamine cycling[3]. [2-^{13}C]acetate injection bolus dosage and infusion was calculated to quickly reach 70% fractional enrichment (FE) (Figure 1C)[4]. MRS experiments were performed on a 14.1T magnet with a 26cm horizontal bore (Agilent/Magnex) using a home-built 9-mm(^{13}C)/15-mm(^{1}H quad) surface coil as transceiver. B_0 inhomogeneity was optimized using first- and second-order shimming with FAST(EST)MAP[5], resulting in water linewidth of 25-30 Hz for a VOI of 68µL (3×5×4.5 mm^3) in the frontal region of the brain (Figure 1D). To characterize the ^{13}C-acetate fractional enrichment (FE) input function and optimize the early detection of glutamate (Glu) and glutamine (Gln) C4 labeling, BISEP-SPECIAL ([6] and references therein) was used for the first 17min (^{13}C-edited and non-edited spectra were acquired in 14 blocks of 8-scans in an interleaved mode (TE/TR=2.8/4000ms)). At later time points where the ^{13}C enrichment in those metabolites gets higher and to measure Glu and Gln C4 and C3 with better spectral separation, it was then interleaved with ^{13}C MRS measured with a semi-adiabatic DEPT polarization transfer sequence (blocks of 128 scans, TR=2.5s, interpulse delay 3.8ms (J_{CH}=130Hz), 45° for last ^{1}H pulse to simultaneously measure signals from CH, CH_2, CH_3 groups)[7]. The interleaved scheme is given in Figure 1B. The last BISEP FE measurement for GluC4 was used to scale the DEPT ^{13}C spectra. BISEP-SPECIAL was also used to measure the initial concentration and time course of the Glu and Gln pools, necessary for the metabolic modelling interpretation. All spectra were frequency corrected and quantified with LCModel[8].

Metabolic Modelling

Two-compartment modeling of [2-^{13}C]acetate (Ace) metabolism was applied to extract metabolic fluxes from the dynamic ^{13}C enrichment curves GluC4, GluC3, GlnC4 and GlnC3, using the measured AceC2 as input function (Figure 2)[3]. Four independent metabolic fluxes were adjusted in this process: the glial tricarboxylic acid (TCA) cycle flux V_g, the transmitochondrial transport V_x, the glutamate-glutamine cycle V_{nt} and neuronal TCA cycle V_{tca}^n. The variance of the obtained metabolic rates was estimated by Monte-Carlo simulations (500 iterations).

Results and Discussion

Despite the low steady-state fractional enrichment achieved when infusing [2-^{13}C]acetate as compared to ^{13}C-glucose and the small acquisition volume achievable in a P30 mouse brain, the quality of the obtained spectra (Figure 1D) enabled both the measurement of MRS-derived metabolic input function (Figure 1C) and ^{13}C time courses for Glu and Gln C4 and C3, both for the control group and GCLM-KO group (Figure 3). Using the proposed two-compartment metabolic model, these four group-averaged labeling curves were successfully described (Figure 4A) and enabled the determination of characteristic oxidative metabolism and glutamate/glutamine cycling rates (i.e. V_g, V_x, V_{nt}, V_{tca}^n, V_{gl}^g and V_{gl}^n) for the control and GCLM-KO groups (Figure 4B and 4C). GCLM-KO mice presented a trend towards lower V_{gl}^g, V_{gl}^n, and V_{nt}, however without reaching statistical significance (two-samples t-test).

We conclude that despite the challenge represented by the low isotopic enrichment resulting from cerebral [2-^{13}C] acetate metabolism and by applying localized dynamic ^{13}C MRS to brains of mice pups, we successfully demonstrated for the first time the measurement of acetate input function and dynamic Glu and Gln labeling, enabling metabolic modelling interpretation in GCLM-KO mice with early postnatal oxidative challenge. This study provides perspectives for studying cerebral metabolism during brain development. Furthermore, GCLM-KO mice presented a trend towards slower oxidative metabolism and glutamate-glutamine exchange, however without reaching statistical significance, which motivates further investigations in a larger sample size.

Acknowledgements

The work was supported by the Center of Biomedical Imaging (CIBM) of the École Polytechnique Fédérale de Lausanne (EPFL), the Université de Lausanne (UNIL), Université de Genève (UNIGE), the Hôpitaux Universitaires de Genève (HUG) and the Centre Hospitalier Universitaire Vaudois (CHUV), the Leenaards, Jeantet Foundations and Biaggi foundation.

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Figures

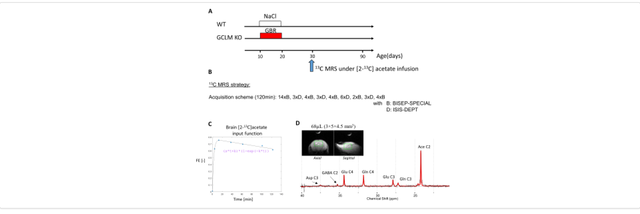


Figure 1: A) Experimental timeline of the GLCM-KO-GBR mouse development and ^{13}C MRS experiment (P30) **B)** Dynamic ^{13}C MRS acquisition strategy. B represents a block of 2x8 BISEP-SPECIAL scans, D represents a block of 128 ISIS-DEPT scans. **C)** ^{1}H-[^{13}C]-MRS derived [2-^{13}C]acetate input function from the measured brain voxel. For the metabolic modelling, the curve was smoothed by fitting it with the function (a·t+b) · (1-exp(-k·t)). **D)** Localization of the ^{13}C MRS 68µl voxel used for both BISEP-SPECIAL and ISIS-DEPT and typical summed ISIS-DEPT spectrum (120min) quantified with LCModel.

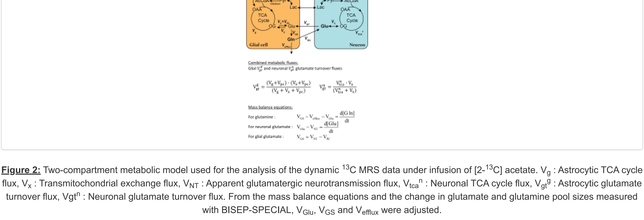


Figure 2: Two-compartment metabolic model used for the analysis of the dynamic ^{13}C MRS data under infusion of [2-^{13}C] acetate. V_g : Astrocytic TCA cycle flux, V_x : Transmitochondrial exchange flux, V_{NT} : Apparent glutamatergic neurotransmission flux, V_{tca}^n : Neuronal TCA cycle flux, V_{gl}^g : Astrocytic glutamate turnover flux, Vgl^n : Neuronal glutamate turnover flux. From the mass balance equations and the change in glutamate and glutamine pool sizes measured with BISEP-SPECIAL, V_{Glu}, V_{GS} and V_{efflux} were adjusted.

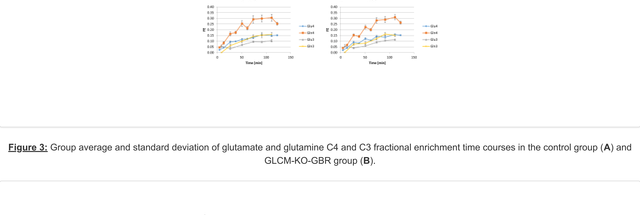
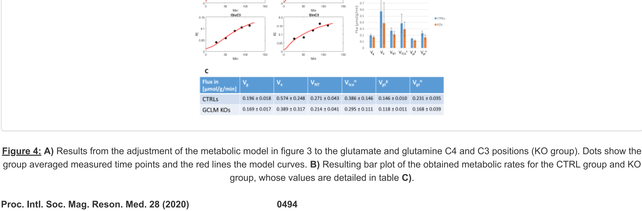


Figure 3: Group average and standard deviation of glutamate and glutamine C4 and C3 fractional enrichment time courses in the control group (**A**) and GLCM-KO-GBR group (**B**).



Flux in [µmol/g/min]	V_g	V_x	V_{nt}	V_{tca}^n	V_{gl}^g	V_{gl}^n
CTRLS	0.196 ± 0.018	0.574 ± 0.248	0.271 ± 0.043	0.386 ± 0.146	0.146 ± 0.010	0.231 ± 0.035
GCLM KOs	0.189 ± 0.017	0.389 ± 0.317	0.214 ± 0.041	0.295 ± 0.111	0.138 ± 0.011	0.168 ± 0.039

Figure 4: A) Results from the adjustment of the metabolic model in figure 3 to the glutamate and glutamine C4 and C3 positions (KO group). Dots show the group averaged measured time points and the red lines the model curves. **B)** Resulting bar plot of the obtained metabolic rates for the CTRL group and KO group, whose values are detailed in table **C)**.