Cerebral activation by fasting results in lactate accumulation in the hypothalamus

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Introduction: Disorders in food intake cause obesity, a multifactorial syndrome that underlies pathologies with largest mortality and morbility in developed countries, including atherosclerosis and ischemic episodes. Food intake is regulated by inhibitory and excitatory signaling systems generated in brain and peripheral organs which have their target at different nuclei situated in the hypothalamus[1]. The delicate balance between these signaling systems determines adequate diet and energetic balance in the body or its alterations. Methods to investigate non-invasively appetite regulation entail, thus, considerable interest in the understanding and prognosis of obesity. Manganese-Enhanced Magnetic Resonance Imaging (MEMRI) has previously shown to be an adequate technique to explore hypothalamic activation in murine models[2]. Manganese is thought to be accumulated in neurons, in a similar way to calcium, revealing zones of neuronal activation[2] (Figure 1). However, little is known on the mechanism of neuronal activation during appetite regulation and how this could affect the neuroglial metabolic coupling mechanisms underlying neurotransmission events. On these grounds we infused (1-^{13}C) glucose in fed and fasted animals, fixed their brains with high power microwaves, dissect the hypothalamic zone and the rest of the brain, and investigated the distribution of the original ^{13}C label between (3-^{13}C) lactate and (4-^{13}C) glutamate, as indicators of glycolytic or oxidative metabolism in these zones. We used a ^{13}C-HRMAS approach to be able to determine ^{13}C labeling in very small brain areas (15-25 mg biopsies) from individual mice. We completed this work by investigating the effects of ghrelin, an orexigenic peptide and Mn^{2+}, on the ^{13}C label distribution from glucose in the dissected regions.

Methods: C57BL/6 male mice (6-8 weeks old) were distributed in four groups (n=5 per group). Animals from groups, 1 (fed animals), 3 (ghrelin-treated animals) and 4 (manganese-treated animals) received water and food *ad libitum*. Mice belonging to group 2 (fasted animals) were fasted overnight, before the experiment. Mice were anesthetized with isoflurane (1%) and groups 1 and 2 received an i.p. injection of (1-^{13}C) glucose (20 μmol/g body weight). Group 3 animals were i.p. injected with ghrelin 0.3 nmol/g body weight, receiving fifteen minutes later an i.p injection of (1-^{13}C) glucose under the same conditions of group 1. Group 4 mice were cannulated in the tail vein for i.v. infusion of $MnCl_2$, receiving 5 μL/g body weight of a 63.2 mM $MnCl_2$ solution at a rate of 0.2 mL/h, followed by an i.p. injection of (1-^{13}C) glucose 15 minutes after the end of the infusion. Fifteen minutes after glucose injection, the metabolism of the mice brain was arrested using a high power (5 kW) microwave fixation system. The brains were then separated in two different regions, hypothalamus and “rest of the brain”. Samples were analyzed in a 11.7 Tesla (500.13 MHz) Bruker AVANCE 500 WB NMR spectrometer using a commercial HRMAS triple ^{1}H, ^{13}C, ^{31}P probe (4 KHz spinning, 4° C). Spectral deconvolution and quantification of relative peak areas were performed with NUTS (Acorn, Freemont, CA, USA). Statistical analysis was performed by Student *t*-test using GraphPad Prism v4.0 (San Diego, CA, USA). Comparisons with $p < 0.05$ were considered statistically significant.

Results: Comparison between the ^{1}H NMR spectra acquired before and after each ^{13}C HRMAS spectrum showed that samples did not suffer significant degradation of the metabolites during the ~15 h necessary to acquire ^{13}C spectra with sufficient signal-to-noise ratio (Figure 2). This is supported by the fact that the relative proportions of N-acetyl-aspartate (NAA) to lactate remained constant during the complete ^{13}C acquisition. We analyzed the incorporation of ^{13}C into the glucose C1α and C1β resonances and the glutamate C4 and lactate C3 resonances, relative to the unchanged natural abundance of ^{13}C inositol C1-C3 resonance. In order to compare the relative changes of (4-^{13}C) Glu and (3-^{13}C) Lac concentrations in each brain zone, we generated a graph (Figure 3) showing the difference in ^{13}C concentration between the “rest of the brain” and hypothalamus. Figure 3 shows that ^{13}C incorporation in Glu C4 is always higher in the “rest of the brain” than in the hypothalamic area. In contrast, the Lac C3 resonance shows that fasted mice depict higher (3-^{13}C) lactate levels in the hypothalamus than in the “rest of the brain”. This indicates that (1-^{13}C) glucose is preferentially transformed to (3-^{13}C) lactate in the hypothalamus of fasted animals, revealing a net increase in glycolysis under hypothalamic stimulation. Neither ghrelin nor Mn2+ could be shown to induce significant changes in the relative concentrations of (3-^{13}C) lactate or (4-^{13}C) glutamate.

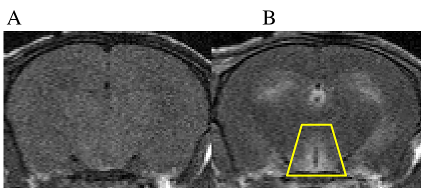

Figure 1. T1-weighted transverse images (TR/TE=600/10 ms) before (A) and after (B) i.v. Mn^{2+} infusion in a overnight fasted mice. Significant enhancement in the hypothalamic zone is shown and highlighted in yellow.

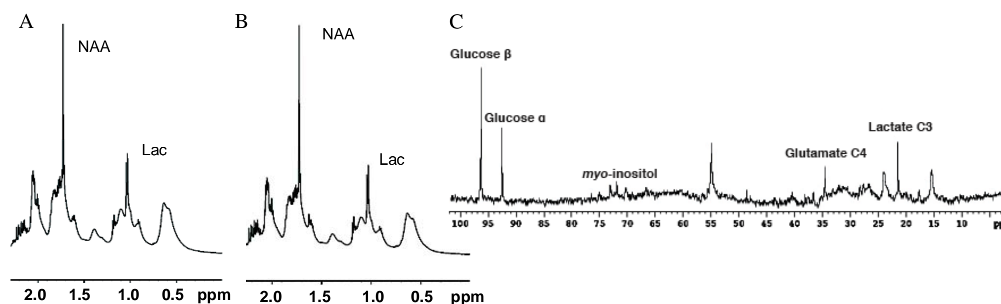

Figure 2. ^{1}H HRMAS spectra from a hypothalamic bioposy before (A) and after (B) the ^{13}C HRMAS acquisition (C). No significant post mortem degradation is observed (compare A and B) during the time necessary for ^{13}C HRMAS.

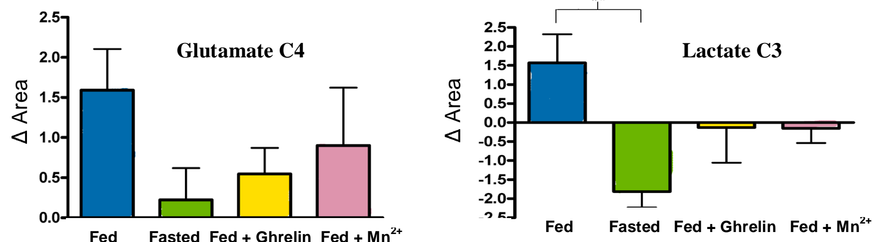

Figure 3. Difference in the ^{13}C incorporation in glutamate C4 and lactate C3 between the “rest of the brain” and the hypothalamic area (Δ Area), for the four groups investigated. Values represent the mean ± SEM (n=5) of glutamate C4 and lactate C3. Data were analyzed using the Student’s *t*-test, using fed animals as the control group; comparisons were made between regions (significantly different from controls: **$p < 0.01$).

Discussion: The alterations described herein indicate that fasting stimulates glycolysis in the hypothalamic zone, precisely the one being highlighted in MEMRI images. This reveal that the fasting stimuli behaves from the point of view of neuroglial coupling, similarly to other sensorial or motor cerebral activation processes[3], albeit probably in a slower time frame. In addition, the finding that ghrelin administration does not mimic the response of the fasted hypothalamus suggest that other additional factors to this hormone, determine the hypothalamic “fasting response” and that Mn^{2+} does not influence the metabolic interactions investigated here. The combination of ^{13}C HRMAS and MEMRI appears as a promising tool to investigate the regional metabolism of the brain during sensorial or motor activation processes.

References: 1. Schwartz, M.W., et al. (2000). *Nature* **404**, 661-671. 2. Kuo, Y.T., et al. (2006). *NMR in biomedicine* **19**, 1028-1034. 3. Maddock, R.J., et al. (2006). *Psychiatry research* **148**, 47-54.