Hypothalamic neuronal activity and intermediary metabolism in ob/ob mice

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**Introduction:** Obesity is a pandemic syndrome, associated with the most prevalent and mortal pathologies in developed countries (Rajala and Scherer, 2003). This pathology results from an imbalance in the control of food intake and energy homeostasis as regulated by a complex network of peripheral and intra-hypothalamic signalling systems (Schwartz et al., 2000). On this basis, the investigation of neuroglial coupling mechanisms and energy metabolism in the hypothalamus is essential to the understanding and integration of the pathways regulating whole body energy homeostasis. Leptin, a cytokine hormone secreted by the adipose tissue, plays an important role in the physiologic regulation of appetite and energy balance by reducing body weight via interaction with hypothalamic neurons (Zhang et al., 1994). In this study, we compared the hypothalamic neuronal activation and intermediary metabolism between wild type (wt) and leptin-deficient (ob/ob) mice through the administration of [1-13C]glucose and applying a regional 13C HR MAS approach.

**Methods:** C57BL/6 (n=6 per condition) and C57BL/6J ob/ob (n=6 per condition) male mice (8-10 weeks old), receiving drinking water ad libitum, were conditioned by feeding (normal chow diet) or fasting (food removed overnight before the experiment) conditions. All mice received an i.p. injection of [1-13C]glucose (20 μmol/g body weight) and, 15 minutes later, cerebral metabolism was arrested using a high-power (5 kW) focused microwave fixation system. The brain was dissected and divided in two areas, hypothalamus and remaining brain. Samples were analyzed in an 11.7 T (125.13 MHz) Bruker AVANCE WB NMR spectrometer (4 kHz spinning, 4 ºC). All 13C HR-MAS resonance areas were normalized to the myo-inositol C1, C3 resonance area, to account for differences in tissue content within the different samples. A two-way ANOVA with Bonferroni’s post-test was applied to compare differences in the metabolites contents between fed and fasted conditions in both mice models. Comparisons with p<0.05 were considered statistically significant.

**Results:** 13C HR MAS spectra obtained from the biopsies allowed the study of the mice cerebral metabolism in hypothalamic and remaining brain. We investigated the incorporation of [1-13C]glucose into lactate (Lac) C3, glutamate (Glu) C4, glutamine (Gln) C4 and GABA C2 resonances, relative to the natural abundance myo-inositol C1, C3 resonance, as represented in Figure 1 for both wt and ob/ob mice in fed (left panel) and fasted (right panel) conditions. In the fed state ob/ob mice show higher Glu C4 and Gln C4 concentrations compared to wt mice (A). Fasting resulted in increased Lac C3 and GABA C2 concentrations in the hypothalamus of both mice (B). Additionally, GABA C2 concentrations increased more in ob/ob mice (A). The Glu and Gln C4 concentrations remained higher for the ob/ob compared to wt (A). Fasted mice of both types have higher Lac C3 concentration in the hypothalamus than fed mice (p<0.05, statistics not shown in the graphs).

**Conclusions:** Increased hypothalamic content of Lac C3 and GABA C2 under fasting conditions occurred in both wt and ob/ob mice showing that this is not a leptin-dependent effect. The higher concentration of these metabolites in the hypothalamus could result from an increased astrocyte-to-neuron lactate shuttle activity and either elevated GABA synthesis, and/or decreased GABA degradation. The higher concentrations of Glu and Gln C4 in ob/ob mice reveal an increased glutamate/glutamine cycle activity and consequently an increased glutamatergic neurotransmission. Taken together, these results suggest that leptin signalling in the hypothalamus may involve, in addition to the well known neuropeptide signalling systems of the hypothalamus, increased glutamatergic neurotransmission.