DWI detected differential response of hypothalamic nuclei between leptin deficient and wild type mice

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PURPOSE: Obesity is a pandemic syndrome associated with the most prevalent and morbid pathologies in developed countries including heart disease, atherosclerosis, diabetes, and cancer[1]. Body adiposity is thought to be regulated systemically through an endocrine 'adiposity'-negative feedback loop, including mainly the adipose tissue-derived leptin[2], which induces anorexigeinc responses after meals. Disruptions in the leptin signalling systems are often associated with obesity in humans and mice, and the leptin-null *ob/ob* mouse model exhibits decrease energy expenditure, hyperphagia and obesity. Previous studies in our lab have shown that leptin-null mice have higher hypothalamic nuclei activation in fed conditions, using MEMRI approaches[3]. On the other hand, we have recently proposed functional diffusion weighted imaging (fDWI) as a new tool to evaluate hypothalamic activation in normal mice and humans[4], avoiding the toxic effects of MEMRI techniques. Here, we extend the fDWI approach to the evaluation of *ob/ob* mice in fed and fasted conditions, to validate the use of fDWI in animal models with hypothalamic disfunctionality and characterize the individual responses to fasting of relevant hypothalamic nuclei from leptin-null mice.

METHODS: *Animal model*: Leptin-deficient B6.V-Lepob/J ob/ob mice (8- to 10-weeks old, n=10), drinking water ad libitum, were imaged in two experimental conditions; fed and after 16h of fasting. Additionally, food and drink intake and activity of a different set of *ob/ob* mice (8- to 10-weeks old, n=8) and control mice (C57 BL6/J, 8- to 10-weeks old, n=8) were measured in metabolic cages during a 72h period time. This period was divided into: 28h of measurements under normal conditions-16h Fasting-28h recovery. *MRI studies:* Mice were anesthetized with 1% isofluorane/oxygen through a nose cap during MRI protocols. We used a 7T Bruker Biospec scanner equipped with a 90mm gradient coil insert (36G/cm) and a mouse head resonator. fDWI was acquired with 4 shot EPI-read gradient and in the L-R, A-P and H-F directions. Acquisition conditions were: δ=4*ms* Δ=20ms, TR/TE=3000/31ms and a 0.164x0.164x1.25mm^3 voxel volume. We obtained 9 high b value acquisitions (300<b<2000 s/mm^2) across an imaging plane containing the hypothalamus (Fig. 1A). Hypothalamic nuclei were selected manually based on the anatomical descriptions given by the mouse brain atlas. *Data analysis:* The data set was analyzed using a biexponential diffusion model[5], described as: $S(b)/S(0)=SDP\cdot\exp(-bD_{slow})+FDP\cdot\exp(-bD_{fast})$, with slow (SDP) and fast (FDP) diffusion phases characterized by slow (D_{slow}) and fast (D_{fast}) diffusion coefficients. Parameter values were obtained by pixel by pixel fitting of the data set using homemade MATLAB v7a libraries.

RESULTS: Measurements in metabolic cages indicated that food and drink consumption were significantly higher in ob/ob mice only under normal conditions. Activity was significantly lower in ob/ob mice in normal conditions and after the fasting period (data not shown). fDWI analyses revealed significantly higher SDP and D_{slow} coefficients in the ventromedial nucleus(VMN) and dorsomedial nucleus (DMN) of *ob/ob* mice in the fed state, when compared to the reported values of C57 mice[4] .The increase of SDP pixel values can be seen in figure 1B as a shift to orange in the ob/ob mice. Figure 2 shows the values of the hypothalamic nuclei with significant differences with control mice. In the fasted state, only the ARC nucleus showed significant differences in the D_{slow} values (not shown). A comparison between feeding and fasting in leptin-null mice revealed significant activation of the arcuate nucleus (ARC) and VMN with fasting, but deactivation in the DMN (not shown).

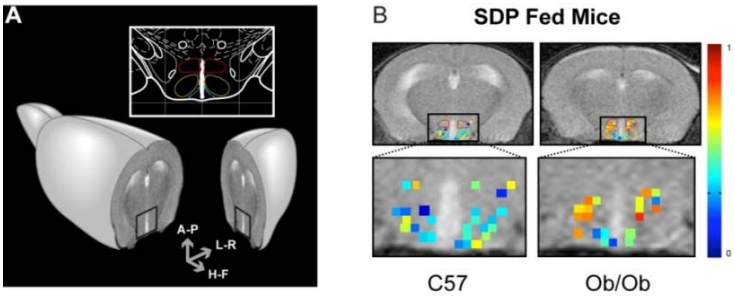

Figure 1. A: Hypothalamic section and localization of the main nuclei, ARC (blue), VMN (yellow) and DMN (red). B: SDP pixel values of representative C57 (upper left) and Ob/Ob (upper right) mice, superimposed to T_2 weighted images and to the nuclei selection. The hypothalamus is enlarged in lower panels.

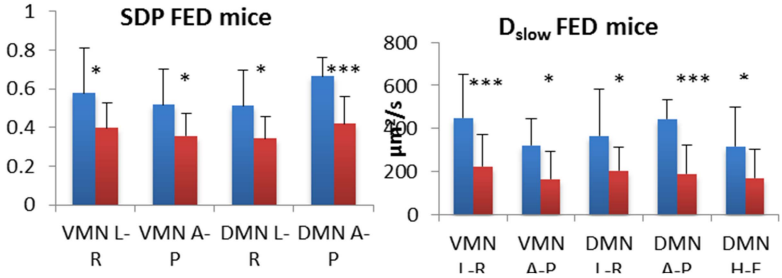

Figure 2. Values (mean±SD) of the nuclei with significant changes of the SDP coefficients (left panel) and D_{slow} (right panel) of fed mice. (t student test, *p<0.05,***p<0.001)

DISCUSSION: fDWI analyses revealed higher hypothalamic activation in the VMN and DMN of the fed Ob/Ob mice compared to the fed values of C57 mice. In fact, fasting increased the diffusion parameter values of C57 mice, virtually to the same levels found in fed ob/ob mice, suggesting that the latter are subjeted to permanent orexigenic stimulation.

CONCLUSION: fDWI reveals significant alterations of the diffusion parameters in individual hypothalamic nuclei as compared to normal lean mice. This method may allow the direct, noninvasive, evaluation of obesity and its response to nutritional or pharmacological interventions.

BIBLIOGRAPHY: Das (2010) [1] Morton et al. (2006)[2] Delgado et al. (2011)[3]Lizarbe et al. (2012)[4]Le Bihan(2006)[5]