fMRI of hypothalamic activation by fasting in ob/ob mice using T2* and fDWI with high and low b values. A comparative study.

Blanca Lizarbe[1], Pilar Lopez-Larrubia[1], and Sebastian Cerdan[1]
[1]*Instituto Investigaciones Biomedicas "Alberto Sols" CSIC-UAM, Madrid, Madrid, Spain*

PURPOSE: Obesity is a pandemic syndrome often associated to 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, mainly supported by leptin[2]. In fact, disruptions in the leptin signalling systems are often associated to obesity in humans and mice, and the leptin-null *ob/ob* mouse model exhibits decreased energy expenditure, hyperphagia and obesity. A variety of neuroimaging tools have been proposed to study appetite regulation in humans and in animal models, including positron emission tomography (PET), and functional magnetic resonance imaging (fMRI)[3]. More recently, we have proposed the use of functional diffusion weighted imaging (fDWI) as a new tool to evaluate hypothalamic activation in normal mice and humans[4]. Moreover, DWI acquisitions at low b values have been successfully used to identify brain activated regions with changes in IVIM perfusion parameters[5]. In this communication, we wish to characterize the activation by fasting in individual hypothalamic nuclei from *ob/ob* mice, using DWI at *high b values*, DWI at *low b values* and T2* imaging, comparing the results obtained with the three techniques.

MATERIALS AND METHODS: *Animal model*: Leptin-deficient B6.V-Lepob/J ob/ob mice (8 to 10-weeks old, n=10, 42 g ±3), drinking water ad libitum, were imaged in two experimental conditions; fed *ad libitum* (regular rat chow) and after 16h of fasting. *MRI studies:* Mice were anesthetized with 1% isofluorane/oxygen during MRI. We used a 7T Bruker Biospec scanner equipped with a 90mm gradient coil insert (36G/cm) and a mouse head resonator. The imaging protocol included successively; first six T_2*-weighted (T_2*w) gradient-echo segmented EPI sequences (TR/TE=182/4ms), followed by two DWI sequences (4 shot EPI, δ=4*ms* Δ=20ms, TR/TE=3000/31ms, L-R, A-P and H-F directions) with 9 *high b* and 7 *low b* values (300<b<2000 and 10<b<150s/mm^2) respectively. All images were acquired across an imaging plane containing the hypothalamus (Fig. 1A) with the same spatial resolution (0.164x0.164x1.25mm^3). Hypothalamic nuclei were selected manually based on the anatomical descriptions given by the mouse brain atlas. *Data analysis:* Each T_2*w image was normalized to the signal intensity of the hippocampus, averaging six acquisitions per animal and rejecting pixels depicting more than 10% variability. The DWI data sets were fitted, to a biexponential model of diffussion[5] (homemade libraries, Matlab v7a): $S(b)/S(0)=SDP\cdot\exp(-bD_{slow})+FDP\cdot\exp(-bD_{fast})$ including slow (SDP) and fast (FDP) diffusion phases with slow (D_{slow}) and fast (D_{fast}) diffusion coefficients. For all investigated parameters, we calculated the mean values in the arcuate nucleus (ARC), ventromedial nucleus (VMN), dorsomedial nucleus (DMN), the sum of three nuclei (ARC+VMN+DMN) and the total hypothalamus.

RESULTS: Figure 1 shows the main results obtained of the investigated parameters. Normalized T_2* signal intensities decreased significantly with fasting in the VMN, DMN, sum of all nuclei and in the hypothalamic region, as depicted in top left panel. Low b DWI showed significant decreases in the SDP in the VMN and sum of all nuclei, and increases in D_{fast} in the overall hypothalamic area (top right panels). High b DWI analyses reported significant increases in SDP coefficients in the VMN, sum of nuclei and hypothalamus, and decreases in the DMN, significant decreases in D_{fast} (lower panels). D_{slow} coefficients increased significantly in all areas investigated except in the DMN (not shown).

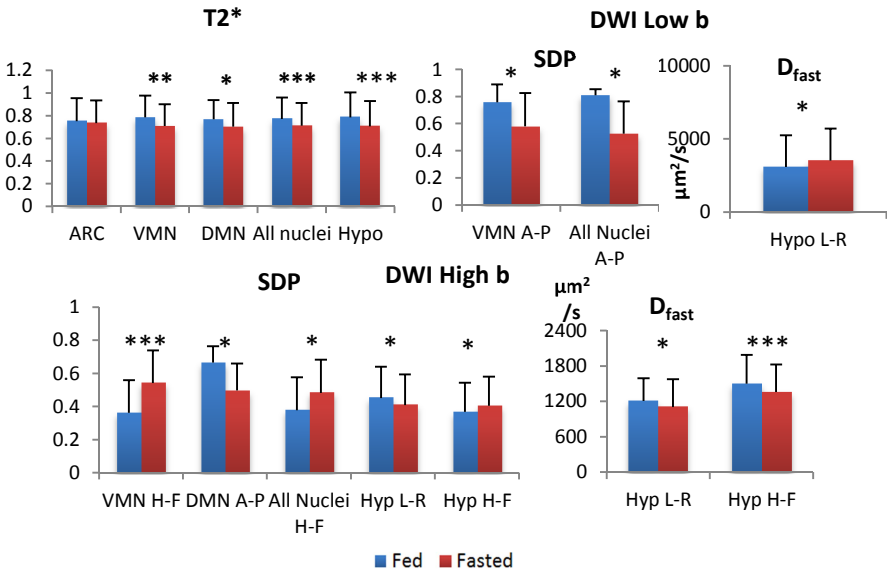

Figure 1. Mean (±SD) values of main investigated parameters and significant differences (t student test, *p<0.05**p<0.005****p<0.001)

DISCUSSION: Present results allow to compare, for the first time to our knowledge, the hypothalamic activation by fasting observed in *ob/ob* mice with three different fMRI techniques; T_2*w, DWI (High b) and DWI (low b). Activation by fasting resulted generally in decreased T2* signal intensities, increased D_{fast} (at low b) and increased SDP (at high b) in ARC, VMN and the overall hypothalamus. These results are consistent with the increases in oxygen consumption and closely circulating deoxihemoglobin (>T_2*), increased blood flow (>D_{fast}/low b) and increased astrocytic swelling derived from augmented orexigenic firing (> SDP/high b). The DMN appeared to depict a different behavior, as previously observed in its MEMRI response, suggesting that the coupling mechanisms between T2* and DWI parameters may differ regionally, probably reflecting a heterogenous perfusion and orexigenic firing within the different hypothalamic nuclei.

CONCLUSION: Taken together, our results suggest that hypothalamic activation by fasting can be observed adequately both with T2* and DWI (high and low b)

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