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

Blanca Lizarbe1, Pilar Lopez-Larrubia1, and Sebastian Cerdan1
1Instituto 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 cancer1. Body adiposity is thought to be regulated systemically through an endocrine ‘adiposity’-negative feedback loop, mainly supported by leptin2. 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 humans3. Moreover, DWI acquisitions at low b values have been successfully used to identify brain activated regions with changes in IVIM perfusion parameters5. 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 T2* sequences (TR/TE=182/4ms), followed by two DWI sequences (4 shot EPI, b=4ms 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<1505/µm²) respectively. All images were acquired across an imaging plane containing the hypothalamus (Fig. 1A) with the same spatial resolution (0.164x0.164x1.25mm³). Hypothalamic nuclei were selected manually based on the anatomical descriptions given by the mouse brain atlas. Data analysis: Each T2* 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 diffusion7 (homemade libraries, Matlab v7a): S(b)/S(0)=SDP·exp(-bD_{low})+FDP·exp(-bD_{fast}) including slow (SDP) and fast (FDP) diffusion phases with slow (D_{low}) 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 T2* 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_{low} (lower panels). D_{low} coefficients increased significantly in all areas investigated except in the DMN (not shown).

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; T2*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 (>T2*), 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 heterogeneous 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)