In vivo MRI/MRS characterization of Brown and White Adipose Tissues in mice: plasticity due to High Fat Diet and pharmacological treatments

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Introduction - Brown adipose tissue (BAT) is currently a topic of interest in obesity and metabolic research due to its physiological relevance in human adults. White adipocytes are characterized by large mono-locular intracellular lipid droplet and reduced cytoplasm, brown adipocytes are endowed with multiple, smaller, intracellular lipid droplets and are extremely rich in mitochondria. Of interest, it has been reported that the water/fat ratio in fat depots is related not only to their morphological structure, but also to BAT metabolic activity. Several works have employed MRS techniques to detect WAT and BAT with respect to the water/fat ratio. Aim of the study was to monitor changes in adipose tissue as a result of high fat diet and after pharmacological treatment with mineralocorticoid receptor antagonists, namely drospirenone (DRSP) and spironolactone (Spiro), in subcutaneous (inguinal) and visceral (perivescical) areas. In our study, MR properties of murine BAT and WAT tissues were assessed in vivo by quantitative ^{1}H MRS analyses, after high fat diet in the presence or in the absence of MR blockade. Moreover, perivescical volume changes were assessed by MRI.

Methods – Female 10 wk old C57bl6 mice were fed with normal chow or a high fat (HF) diet for 12 weeks. Mice fed HF were concomitantly treated for 12 weeks with either vehicle, DRSP or Spironolactone, administered as subcutaneous pellets, releasing 6 mg/Kg/day (DRSP) or 20 mg/Kg/day (Spiro). During this period body weight and different metabolic parameters were measured. After 12 weeks the assessment of fat composition in perivescical and inguinal fat was performed by 1H MRS and quantification of perivescical fat was assessed by MRI analyses.

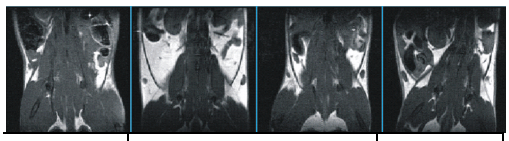
MRI and MRS experiments were performed on mice using a Varian INOVA MRI/MRS system (Varian, Palo Alto, USA) operating at 4.7 T with a transmitter volume RF coil actively decoupled from the receiver surface coil (RAPID Biomedical, Rimpar, Germany). Mice were anaesthetized by Sevoflurane 3.0 % in O_2, 1 L/min.

Coronal (TR/TE = 600/18 ms, 4 transients, 23 slices, thickness = 0.8 mm, FOV 50 x 35 mm^2, matrix 256 x 128) and axial multislice spin echo images (TR/TE = 700/18 ms, 4 transients, 29 slices, thickness = 0.8 mm, FOV 35 x 30 mm^2, matrix 256 x 128) were acquired from the abdomen for quantitative evaluation of visceral fat volume. Images were analyzed using the Varian VNMRJ 1.1D software.

A STEAM sequence with an inversion recovery pulse was applied to voxels positioned in the inguinal (close to the lymphonode) and perivescical fat depots. STEAM spectra were acquired at progressively longer TEs of 9, 13, 18, 22, 28 and 33 ms for T2 determination. Spectra were then collected at minimum TE of 9 ms and TIs of 50, 100, 200, 300, 400, 600, 1000, 2000 and 6000 ms for T1 evaluations. STEAM spectra with TR/TE/TM=6000/9/10 ms have been used for water and lipid quantitative determination. LCModel was used for spectral fitting.

Histological analysis of subcutaneous and visceral fat have been also performed.

Results – MRI experiments showed significant differences in the perivescical volume of all the groups (ANOVA, P<0.01) (see Figure 1). Multiple posthoc comparisons (Fisher test) evidenced a significant difference between the volumes of DRSP- (n=6) compared with HF-treated (n=5) (P=0.02) and Spiro- (n=6) compared with HF-treated (n=5) (P=0.01) with a trend towards ND group, with a marked increase in white fat volume induced by HF diet, which was countered by MR antagonism. This was in accordance with macroscopic and microscopic analysis



ND	HF	HF+DRSP	HF+Spiro
442 ±156	1648 ± 339	840 ±393	899 ± 250

Figure 1- Perivesical volumes are expressed in mm^3

MRS analyses on fat depot showed significant differences in the water over CH_3 lipid signal at 0.9 ppm among the high fat diet groups (ANOVA, p= 0.01) in inguinal but not in perivescical fat (Figure 2). Multiple posthoc comparisons (Fisher test) evidenced a significant difference between the wat/lip0.9 ratio of DRSP- (n=3) compared with HF-treated (n=4) (P=0.006) and Spiro- (n=3) compared with HF-treated (n=4) (P=0.01).

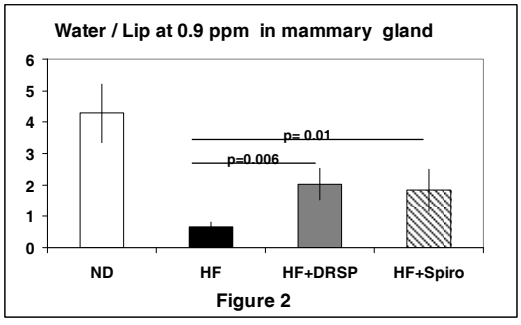


Figure 2

Also the percentage of water was significantly increased in the inguinal fat of DRSP- and Spiro- (p= 0.001 and p= 0.02, respectively) compared with HF-treated animals, suggesting an increase in brown-like adipocytes, richer in water than white adipocytes

Histological analysis showed a marked increase in size of white adipocytes induced by high fat diet, which was countered by concomitant treatment with mineralocorticoid receptor antagonists. Interestingly, treatment with DRSP and Spiro markedly increased the number of brown-like adipocytes interspersed in all depots examined, suggesting active "browning" of adipose tissue.

Discussion and Conclusions – We report the evidence by in vivo 1H MRS to monitor differential tissue composition between WAT and BAT in fat depots, and to study volume alterations due to HF diet and concomitant pharmacological treatments with mineralocorticoid receptor antagonists. Our results demonstrate that MRI/MRS can be used to monitor changes in fat deposit to evaluate effects of drugs in rodent models of obesity.

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