In vivo characterization of dietary effects and drug efficacy on fat accumulation in liver, abdomen and skeletal muscle

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Introduction: Obesity is a medical condition contributing to several health problems such as cardiovascular disease and type 2 diabetes, therefore there is a need to discover and develop new anti-obesity drugs. Current measurements to evaluate the anti-obesity effects are focused on the decrease of the body weight and visceral fat which takes longer time to show the effect, therefore a biomarker to predict anti-obesity effects for short term is of interest. In this study, we investigated the change of intra-hepatic lipid (IHL), intra-myocellular lipid (IMCL) and visceral fat in addition to the change of body weight, in a series of studies using calorie restriction and an anti-obesity drug (Sibutramine) treatment for 4 weeks in a diet induced obese rat model (DIO) (Fisher male rat, Clea Japan).

Methods: Control group (n = 5) received control diet (Clea Japan, CE2) from 4 weeks of age. High fat diet group (n = 15) were fed with high calorie diet (Research Diet, D12079B) from 4 to 17 weeks of age to induce obesity and insulin resistance. After 17 weeks, control group continued the same CE2 diet and the high fat diet group was further divided into 3 subgroups: (i) RD group (n = 5) continued the same high fat diet, (ii) Calorie restriction (CR) group (n = 5) was fed the same high fat diet with 20 % reduction in calorie intake and (iii) Sibutramine (Sib) group (n = 5) was given high fat diet and sibutramine (3 mg/kg, 5ml/kg of saline) for 4 weeks (Day 0-28). Sibutramine suppresses appetite by inhibiting the reuptake of the neurotransmitters norepinephrine and serotonin. During this period of 4 weeks, body weight, food intake, blood glucose, triglyceride, cholesterol, insulin and leptin levels were measured along with liver fat, visceral fat and IMCL. The estimation of visceral fat accumulation and the measurement of body fat composition were performed by MRI/MRS scanning. MRI and MRS measurements were performed on a 7 Tesla Bruker ClinScan using volume transmit and surface receive coils in abdomen, liver and skeletal muscle (tibialis posterior) on Day 1, 3, 8, 15 and 29 of the treatment. Fat estimation in the abdomen was based on axial image segmentation. Abdomen imaging was performed by T_2 weighted spin echo sequence with a FOV of 55 x 55 mm and matrix size of 256 x 256. Abdomen images in the coronal plane were used as reference and water suppressed transverse images from L1 to L5 of the spine was acquired. Segmentation of visceral and subcutaneous fat was performed using graph theoretical method [1] (Figure 1) by a custom-developed MATLAB program. Localized PRESS experiments were performed on liver and skeletal muscle with a voxel size of 4 mm^3 and 3 mm^3, respectively with TR = 4.0 sec, TE = 13 msec (Figure 2). Liver fat estimation was based on spectral analysis by LCModel. The % liver fat was calculated using concentrations of lipid methyl, methylene and the water signal [2]. The unsuppressed water signal was employed for eddy current correction. LCModel fitting of skeletal muscle spectrum was used to estimate levels of intra-myocellular lipid (IMCL).

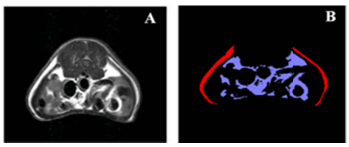

Figure1. (A) Transverse image of rat abdomen (B) segmented subcutaneous (red) and visceral adipose tissue (blue).

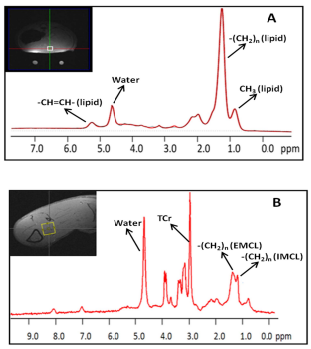

Figure 2. Localized 1D PRESS spectrum of (A) liver and (B) skeletal muscle.

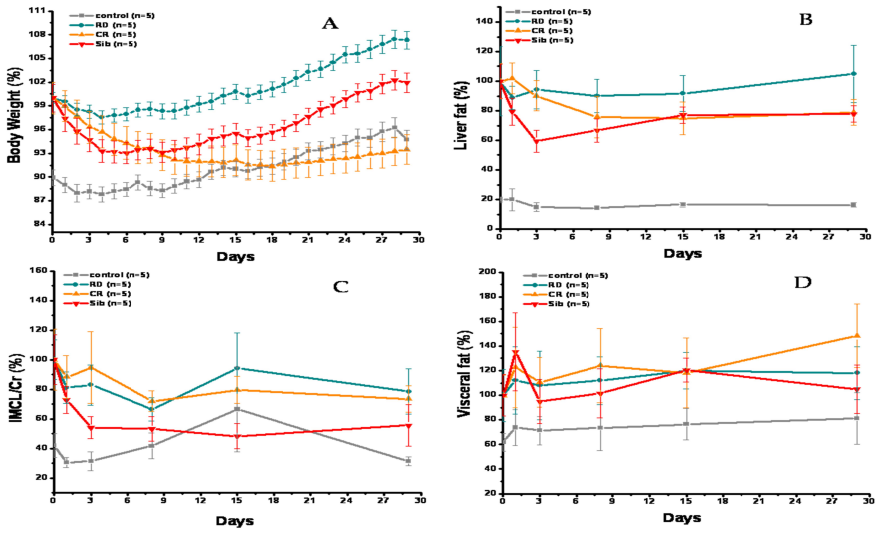

Figure 3. (A) Body weight from Day 0-29. (B) Total % fat in liver, (C) intra-myocellular lipid (IMCL/Cr) concentration in skeletal muscle (tibialis posterior) and (D) visceral fat through Day 0-29. The gray line: control group (CE2 diet), green line: RD group, orange line: CR group and the red line: Sib group. The data of high fat diet groups on Day 0 are normalized to 100 %.

Results and Discussion: *Figure 3A (body weight):* The body weight changes in the control and RD groups were similar. They showed a minimal change up to Day 8, and then had a gradual increase up to the end of the study. On the other hand, CR group showed a gradual decrease due to calorie restriction up to day 15. Sib group showed a sharp decrease at the beginning of treatment and then increased gradually after Day 8. *Figure 3B (Liver fat):* RD, CR and Sib groups showed higher liver fat value than control group due to high fat diet. Control and RD group showed minimal variation over the period of 30 days. On the other hand, CR group showed gradual decrease up to day 8 and Sib group showed a sharp decrease up to Day 3. The changes of CR and Sib groups had a similar trend of change in the body weight in the study period. *Figure 3C (IMCL/Cr):* RD, CR and Sib groups also showed higher IMCL/Cr value than control group due to high fat diet. RD and CR group showed minimal variation in IMCL levels. Sib group showed an acute decrease at the beginning of the treatment and was steady up to the Day 29. *Figure 3D (Visceral fat):* In control, RD and CR groups, there were no significant changes. On the other hand, a sharp increase on Day 1 and then further reduction during Day 3-8 were seen in Sib group.

Conclusion: We have studied the variation in liver fat, skeletal muscle fat and visceral fat with diet restriction and sibutramine treatment. A transient response of 4 weeks sibutramine treatment was monitored in the obese rat model and this response was different from other groups. Our findings demonstrate that MRI/MRS can be utilized to follow the adiposity deposit in organs including abdomen, liver, and skeletal muscle and the approach can be used to evaluate the therapeutic potential of novel drugs in rodent models of obesity and diabetes.

Reference

[1] Suresh et al. NeuroImage, 49, 225-239 (2010). [2] Cowin et al. JMRI, 28, 937-945 (2008).