MR imaging and spectroscopic investigation of exercise and calorie restriction in high fat diet fed obese rats

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Objective: Obesity is a medical condition contributing to major health problems including cardiovascular disease and type II diabetes in both developed and developing countries. There is an immediate need for reducing obesity by physical exercise, calorie restriction and anti-obesity drugs. Our study aims to investigate the influence of exercise intervention and calorie restriction on obesity parameters, employing magnetic resonance imaging and spectroscopy (MRI/MRS). We have measured the changes in liver fat, intra-myocellular lipid (IMCL) in skeletal muscle, visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) in a series of studies using exercise intervention and calorie restriction with diet induced obese (DIO) rat model (male Fischer 344 rat, Clea Japan).

Methods: Obesity was induced in rats by high fat diet (Research Diet, D12079B) from 5 to 18 weeks of age, all rats were divided into 5 groups (n = 7): control (Ct), exercise once a day (Ex1) and exercise twice a day (Ex2), and calorie restriction with -15% (CR15) and calorie restriction with -30% (CR30) reduction of total calorie intake compare with control group. Each exercise duration was 30 min (20m/min) using the treadmill (Columbus-1055SRM-E54 Exer-3/6-Dual). The intervention period was 4 weeks. The body weight and food intake was measured. Blood parameters (glucose, triglyceride, insulin, leptin) were measured on pre- and post-intervention, and insulin resistance index was calculated using a formula: fasting glucose (mg/dL) x fasting insulin (μU/mL) / 405. Liver fat, IMCL and visceral/subcutaneous fat volume were measured by 7T Bruker ClinScan MR System. T_2 weighted spin echo sequence with a FOV (65 x 65 mm) and matrix size of 256 x 256 was used for acquiring the abdomen images. Coronal slices were acquired for localizing the L1-L5 region of the spine for reference and water suppressed transverse images were used for segmentation and quantitation of fat. Segmentation of visceral and subcutaneous fat was performed using a hybrid segmentation (Region based active contours – RAC and Fuzzy C-means - FCM) method [1, 2] (Figure 1) by a in-house developed MATLAB® program. The subcutaneous and visceral fat regions were differentiated using the RAC and for the segmentation and FCM was employed for quantification of fat. Localized PRESS based MR Spectroscopy 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 = 14 msec (Figure 2). Quantification of liver fat was calculated using concentrations of lipid methyl, methylene, water signal [3] and LCModel® based spectral analysis. The skeletal muscle IMCL were obtained from the linear combination model based spectral analysis. Eddy current correction for spectral analysis was done using the unsuppressed water signal.

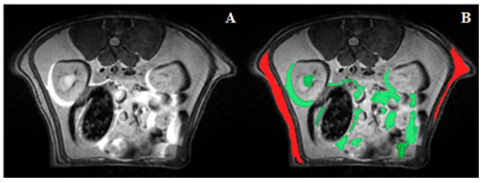

Figure1. (A) Transverse image of abdomen, (B) Overlay of the segmented visceral (green) and subcutaneous (red) adipose tissue.

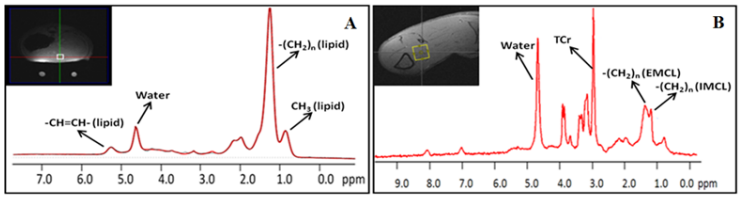

Figure 2. Localized 1D PRESS spectrum of (A) liver and (B) skeletal muscle.

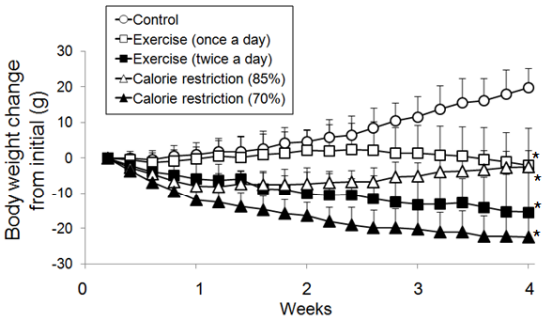

Figure 3. Longitudinal body weight change (g). Data are shown as mean + SD. Body weight change is normalized to the average value of each group on Day 1 (362-367 g). * $P < 0.05$ vs. Ct group on Day 28.

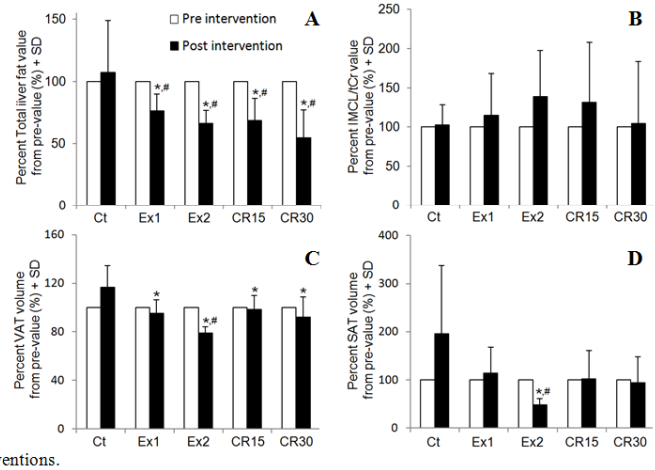

Figure 4. (A) Total liver fat, (B) IMCL/Cr (C) VAT and (D) SAT volumes. Post values are calculated as percentage to normalized pre values of 100% and are shown as mean + SD. *$P < 0.05$, vs. post-intervention value of Ct group. #$P < 0.05$ vs. pre-intervention value of each group.

Table 1. Blood parameters and insulin resistance index of pre- and post interventions.

		Plasma TG (mg/dL)		Plasma Leptin (ng/mL)		Plasma glucose (mg/dL)		Plasma insulin (μU/mL)		Insulin resistance index	
	Group	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
4 weeks	Ct	434 ±100	473 ±56	22 ±4.5	34 ± 6.5	156 ±14	159 ±10	114 ±22	97 ±18	44 ±5.4	38 ±7.9
	Ex1	454 ±80	498 ±142	23 ±3.0	18 ± 2.6#,*	164 ±17	165 ±25	114 ±14	71 ±14#,*	47 ±9.4	29 ±5.9#
	Ex2	501 ±175	563 ±249	26 ±7.4	15 ± 3.7#,*	182 ±19	160 ±21#	121 ±32	68 ±32#,*	54 ±14	27 ±15#
	CR15	463 ±118	459 ±76	27 ±4.8	27 ± 4.9*	177 ±26	159 ±15	140 ±50	89 ±34#	62 ±26	35 ±14#
	CR30	507 ±144	425 ±70	24 ±6.9	21 ± 6.9*	165 ±14	163 ±19	106 ±29	82 ±19	44 ±14	33 ±6.2

*$P < 0.05$, vs. post-intervention value of Ct group, #$P < 0.05$ vs. pre-intervention value of each group.

Results: Exercise intervention and calorie restriction provided body weight reduction compared to the rats on high fat diet (Figure 3). Rats which had exercised interventions twice (Ex2) and calorie restriction (30%) CR30 groups showed significant decrease in body weight (Figure 3). All Ex and CR groups showed significant decrease in total liver fat compared to the control group on high fat diet which had increase in liver fat (Figure 4A). Increase in IMCL/Cr was observed with 28 days of exercise intervention whereas no change was observed with calorie restriction (CR30)(Figure 4B). Significant decrease in VAT (Figure 4C) and SAT (Figure 4D) was observed due to exercise interventions. The calorie restriction decreased the VAT significantly compared to SAT (Fig 4C and Fig 4D). The plasma leptin of all Ex and CR groups were significantly decreased compared to that of Ct group. A significant decrease of plasma insulin was also seen in Ex groups (Table 1).

Conclusion: We have quantitatively assessed the changes in fat metabolism with exercise and calorie restriction using MRI and MRS techniques in high fat diet fed rat model. Our preliminary results demonstrate the changes in the fat patterns (abdomen, liver and muscle) for both calorie restriction and exercise interventions.

References : [1] Shawn Lankton et al. *IEEE Trans on Image Processing*, 17, 2008, 2029-39. [2] Bezdek JC et al. *Computers & Geosciences*, 10, 1984, 191-203. [3] Cowin et al. *JMRI*, 28, 2008, 937-945.