Modulation of the abdominal and hepatic fat by adipose-specific fat-storage inducing transmembrane2 (FIT2) protein

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TARGET AUDIENCE: Researchers interested in studying lipid mobilization due to the modulation of FIT protein expression

PURPOSE: The unique ability of adipose tissue to expand and to store triglycerides in cytosolic lipid droplets is essential in mammalian organisms to maintain energy homeostasis. Triglyceride (TAG) rich unilocular lipid droplets (LDs) in white adipose tissue (WAT) serve as the main storage depot in the body for energy, and particularly when energy intake exceeds that of energy expenditure. Under the conditions of excessive energy intake, triglyceride accumulation can lead to obesity and associated diseases such as type 2 diabetes, cardiovascular disease, and other metabolic syndromes[1]. Over expression of fat storage-inducing transmembrane (FIT) proteins results in the accumulation of triglycerides. Knockdown or down regulation of FIT2 protein in animal models can be utilized to evaluate the targeted therapeutic interventions. In our current study we have investigated the effect of adipose specific fat storage-inducing transmembrane 2 (AFIT2) protein modulation on abdominal and hepatic fat depots in both FIT2 adipose-specific knockout (AFIT2KO) and their littermate (LL) groups.

ANIMAL MODEL AND MR METHODS: Adipose-specific FIT knock out (AFIT2KO) n=5 and their littermate controls (n=5) mice were subjected to imaging experiments as approved by institutional animal care and use committee. The "floxed" FIT2 mice were generated by flanking exon 1 of FIT2 with LoxP sites through gene-targeting. Later floxed FIT2 alleles were crossed to aP2-cre mice to generate adipose-specific FIT2 knockout mice (named as AFIT2KO). Mice having both FIT2 alleles flanked with loxP sites (littermate: LL) were used as controls. In vivo MRI and MRS experiments with motion compensated respiratory gating were performed on a 7 T ClinScan MRI/MRS scanner (Bruker) equipped with a 72-mm volume resonator, in combination with a 20-mm surface receive-only coil. A volume-localized PRESS sequence was employed on liver with TR=4 s, TE= 13 ms, number of averages NA 128, voxel volume=64 mm^3, spectral width= 3500 Hz, with 2048 complex points per free induction decay. Fat content from in vivo spectra were estimated using LC Model software[2]. Dixon imaging was performed in the transverse plane to acquire fat and water images from the abdomen region (lumbar I to V) with TR- 8 ms, AV- 2, slices- 22 (1 slab), TE(opposite phase)- 1 ms, TE(in-phase)-2.5 ms, flip angle- 60, BW-1090 and 1500 Hz/pixel, in-plane resolution-0.195x0.195 mm and slice thickness- 1mm. Segmentation of subcutaneous adipose tissue (SAT) fat and visceral adipose tissue (VAT) fat was performed using a hybrid algorithm (levelset[3-4] and fuzzy c-means –FCM) by an in-house developed MATLAB program. High frequency noise was filtered from dixon data using anisotropic filter while preserving the edges. Strength of the edges in the image volume was improved using edge enhancement techniques. The distance regularized evolution based level-set was allowed to expand and contract for localizing the boundary between SAT and VAT. FCM, with different number of classes, was performed on SAT and VAT regions respectively and thresholds for classifying the data into fat and non-fat regions were derived using the statistical parameters. Quantitation of SAT and VAT regions was performed using the voxel dimensions of images.

RESULTS: Figure 1A and 1B show the SAT (red) and VAT (orange) fat fractions from the segmented image of the abdomen region from AFIT2KO and Littermate mice respectively. **Figure 2A, B** shows the fat content in subcutaneous and visceral regions of LL and AFIT2KO mice. Both SAT and VAT fat contents of AFIT2KO mice were significantly ($P < 0.05$) lower than LL mice. In AFIT2KO mice the total volume of the SAT content (509 ± 13 mm^3) in lumber I to V was significantly ($P < 0.05$) lower compared to AFIT2KO mice (575 ± 27 mm^3). Volume of VAT content (723 ± 29 mm^3) in AFIT2KO was also significantly ($P < 0.05$) lower compared to LL mice (907 ± 48 mm^3). **Figure 3** show the stacked spectra of liver from AFIT2KO and LL mice. Lipid resonances in liver spectra were dominant in AFIT2KO mice compared to liver spectra of LL mice. **Figure 4** show the fat fractions of liver from AFIT2KO and LL mice. The liver fat fraction in AFIT2KO mice (0.040) was significantly ($P < 0.05$) higher than LL mice (0.014). **Figure 5A, B** show the oil red O and hematoxylin & eosin (H & E) staining of AFIT2KO and LL mice at 20X magnification.

Figure 1

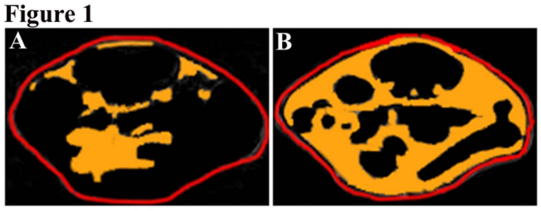


Figure 2A, B

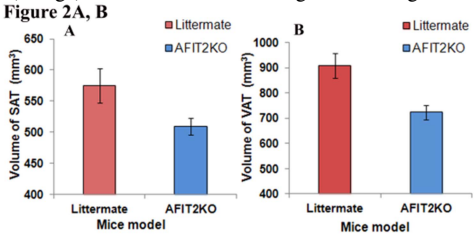


Figure 3

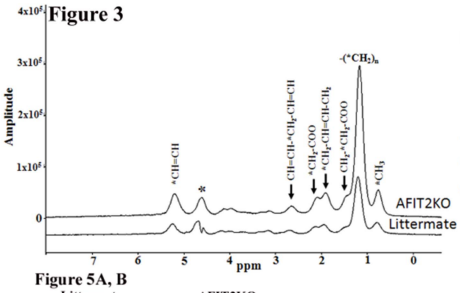


Figure 4

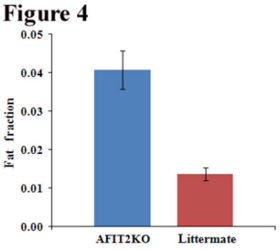
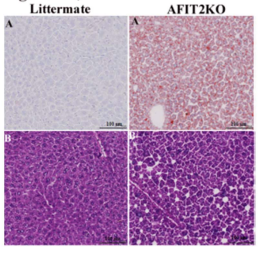


Figure 5A, B



DISCUSSION: Volumes of SAT and VAT fat fractions were significantly ($p < 0.05$) higher in LL control mice compared to AFIT2KO mice whereas hepatic fat fraction was significantly higher in AFIT2KO compared to LL mice indicating a vital role of FIT2 in lipid accumulation. Silencing the FIT2 protein specifically reduced the fat accumulation in the adipose tissue only. Knockdown of FIT2 protein resulted in enhanced liver fat accumulation in FIT2 knockout mice compared to littermate control group. It is interesting to note that silencing FIT2 protein resulted in specifically reducing the fat accumulation in adipose tissue there by reallocating the triglycerides from adipose to liver. Accumulation of fat in the liver was further confirmed by oil red O and hematoxylin & eosin (H & E) staining. The fat deposition (lipid droplets) in stained sections of the AFIT2KO animals is of mixed type (macro- and micro vesicular patterns).

CONCLUSIONS: In vivo results indicated that the silencing of the AFIT2 protein has specifically reduced the fat accumulation in adipose tissue whereas increased the liver fat in AFIT2KO mice. Controlling the expression of adipocyte specific or hepatocyte specific FIT2 proteins by intervening with targeted drugs can be explored to mobilize the fat accumulated in the abdominal and liver in obesity and type 2 diabetic conditions.

REFERENCES: (1). Savage D.B. et.al., Physiol Rev. 87: 507, 2007. (2). Provencher et. al., NMR Biomed. 14: 260, 2001. (3) C. Li, et. al., IEEE Trans. Image Process, 12: 3243, 2010. (4) Lankton S., Tannenbaum A., IEEE Trans. Image Processing, 17: 2029, 2008.