Modulation of the abdominal visceral and subcutaneous adipose tissue by fat storage-inducing transmembrane (FIT) proteins - An Imaging Study

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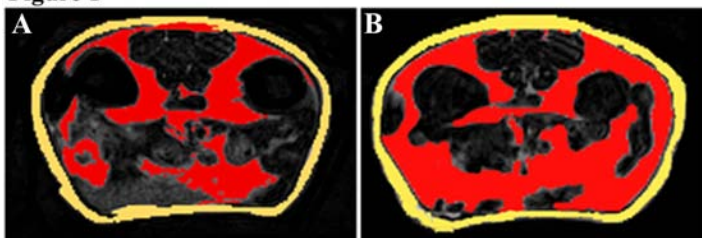
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Target Audience: Researchers interested in studying the fat metabolism using FIT2 adipose-specific knockout mice models
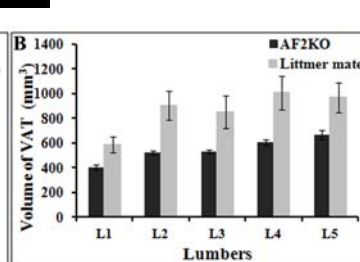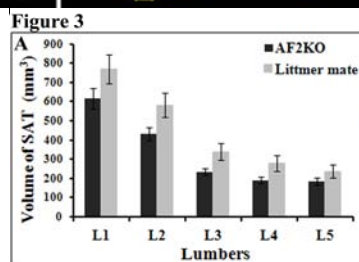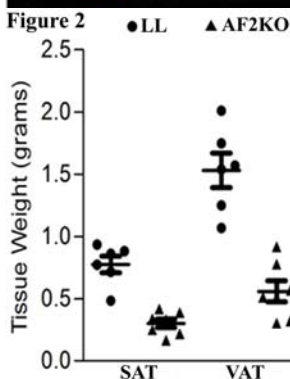
Introduction: Adipose tissue stores triglycerides in cytosolic lipid droplets that are essential for maintaining energy balance at the cellular and whole organism level under normal physiological conditions. Lipotoxicity due to excessive accumulation of triglycerides results in insulin resistance, type 2 diabetes mellitus and increased risk of cardiovascular diseases[1]. Fat storage-inducing transmembrane (FIT) proteins are localized in endoplasmic reticulum (ER) and their over expression results in the accumulation of triglycerides. Fat accumulation in the body can be controlled by silencing the FIT2 protein in *in vivo* animal models and can be utilized to evaluate different therapeutic interventions including drugs, exercise and calorie restriction. In our current study we have evaluated the abdominal fat in both FIT2 adipose-specific knockout (AF2KO) and their littermate (LL) control mouse models using magnetic resonance imaging.

Animal model and MR imaging: Adipose-specific FIT knock out (AF2KO) n=6 and their littermate controls (n=6) mice were subjected to imaging experiments as approved by institutional animal care and use committee. We used gene-targeting to generate viable "floxed" FIT2 mice by flanking exon 1 of FIT2 with LoxP sites. Mice carrying floxed FIT2 alleles were crossed to aP2-cre mice to generate adipose-specific FIT2 knockout mice, henceforth named AF2KO. Littermates having both FIT2 alleles flanked with loxP sites (LL) were used as controls and were fed a 60% high fat diet (HFD) for 12 weeks prior to imaging. In-vivo magnetic resonance imaging (MRI) experiments with motion compensated respiratory gating were performed on 7T ClinScan MRI scanner equipped with a 40 mm diameter mouse body volume coil resonator (transmit and receive). Lumber I (L1) to lumber V (L5) of abdomen region were localized by T2 weighted coronal MR imaging. Dixon imaging was performed in the transverse plane to acquire fat and water images from the abdomen region (lumber I to V) with TR- 8 ms, averages- 2, slices- 22 (1 slab), echo times (TE) opposite-phase-1 ms, TE in-phase-2.5 ms, flip angle- 60, echo bandwidths-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 (level-set[2-3] and fuzzy c-means –FCM[4]) 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.

Figure 1

Results and Discussion: Figures 1A and 1B show the SAT (yellow) and VAT (red) fat fractions from the segmented image of the abdomen region from AF2KO and littermate mice respectively. In LL mice the total volume of the SAT fat in lumber I to V was 2206 ± 245 mm^3 compared to AF2KO mice (1655 ± 127 mm^3). Volume of VAT fat in LL and AF2KO mice were 4317 ± 544 mm^3 and 2722 ± 67 mm^3. Volumes of both SAT and VAT fat fractions were significantly ($p < 0.05$) higher in litter mate compared to AF2KO mice. After the MRI experiments, animals were euthanized and collected the SAT and VAT fat depots to perform the fat

Figure 2

Figure 3

pad measurements. Average fat content in subcutaneous and visceral regions of LL and AF2KO mice are shown in **Figure 2**. Both SAT and VAT fat contents of AF2KO mice were significantly ($p < 0.05$) lower than LL mice, indicating a vital role of FIT2 in lipid accumulation. Silencing the FIT2 protein has reduced the fat accumulation even in high fat diet-fed conditions. It has also been reported that knockdown of FIT2 in zebrafish or 3T3-L1 adipocytes results in significant decrease in lipid droplet formation[4]. Further, in the current study to understand the deposition of the fat around the individual lumbers (L1, L2, L3, L4 and L5) of the abdomen, we performed lumber wise fat analysis of corresponding imaging data. **Figure 3A and 3B** shows the distribution of average SAT and VAT across L1 to L5 in AF2KO and LL mice. In both groups of mice the SAT distribution (**Fig 3A**) in L1 and L2 regions (upper part of the body) contributed largely to the total SAT content. The volume of the SAT content of littermate control mice in L1 and L2 regions was significantly ($P < 0.05$) higher compared to AF2KO mice. In **Fig 3B**, deposition of the VAT in littermate control mice was significantly ($P < 0.003$) higher in L2 to L5 (whole abdomen) compared to L1 region. In AF2KO mice the major volume of VAT is distributed in L4 and L5 regions. Across L1 to L5, the total SAT and VAT fat content was significantly ($P < 0.05$) higher in littermate control mice compared to AF2KO mice.

Conclusion: FIT2 knockout mouse model with HFD intervention had significantly lower amount of fat content compared to litter mate mice. Silencing the FIT2 protein has reduced the excessive lipid accumulation. Modulation (either down regulation or silencing) of these proteins by drugs in obesity and type 2 diabetic conditions might be helpful in preventing the fat accumulation in the body. **References:** (1) Spiegelman BM, Flier JS., (2001) Cell, 104:531–543. (2) C. Li, et. al., (2010) IEEE Trans. Image Process, 12, 3243-54. (3) Lankton S., Tannenbaum A., (2008) IEEE Trans on Image Processing, 17, 2029-39. (4) Kadereit B., et. al., (2008) Proc Natl Acad Sci USA, 105, 94-99.