Comparison of brown and white adipose tissue fat fractions in ob, seipin, and Fsp27 gene knockout mice by chemical shift-selective imaging and \(^1\)H-MR spectroscopy

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Peng X, Ju S, Fang F, Wang Y, Fang K, Cui X, Liu G, Li P, Mao H, Teng G. Comparison of brown and white adipose tissue fat fractions in ob, seipin, and Fsp27 gene knockout mice by chemical shift-selective imaging and \(^1\)H-MR spectroscopy. Am J Physiol Endocrinol Metab 304: E160–E167, 2013. First published November 13, 2012; doi:10.1152/ajpendo.00401.2012.—Brown adipose tissue (BAT) plays a key role in thermogenesis to protect the body from cold and obesity. White adipose tissue (WAT) stores excess energy in the form of triglycerides. To better understand the genetic effect on regulation of WAT and BAT, we investigated the fat fraction (FF) in two types of adipose tissues in ob/ob, human BSCL2/seipin gene knockout (SKO), Fsp27 gene knockout (Fsp27\(^{−\text{/−}}\)), and wild-type (WT) mice in vivo using chemical shift selective imaging and \(^1\)H-MR spectroscopy. We reported that the visceral fat volume in WAT was significantly larger in ob/ob mice, but visceral fat volumes were lower in SKO and Fsp27\(^{−\text{/−}}\) mice compared with WT mice. BAT FF was significantly higher in ob/ob mice than the WT group and similar to that of WAT. In contrast, WAT FFs in SKO and Fsp27\(^{−\text{/−}}\) mice were lower and similar to that of BAT. The adipocyte size of WAT in ob/ob mice and the BAT adipocyte size in ob/ob, SKO, and Fsp27 mice were significantly larger compared with WT mice. However, the WAT adipocyte size was significantly smaller in SKO mice than in WT mice. Positive correlations were observed between the adipocyte size and FFs of WAT and BAT. These results suggested that smaller adipocyte size correlates with lower FFs of WAT and BAT. In addition, the differences in FFs in WAT and BAT measured by MR methods in different mouse models were related to the different regulation effects of ob, seipin, or Fsp27 gene on developing WAT and BAT.

Although BAT plays a significant role in preventing obesity and insulin resistance (3), it has been shown that the accumulation of excess WAT contributes to the development of cardiovascular, metabolic, and renal disorders (31, 33). Rodents and other small mammals have copious BAT throughout their lifespans, but recent evidence has suggested that BAT is also present in humans. This finding has led to a growing interest in understanding the development and physiology of BAT (8, 36).

Animal models with selective gene knockout have improved our understanding of the pathogenesis of obesity and lipid metabolism disorders. Further efforts in studying mouse models will help to further understand the pathogenesis of BAT and WAT, which will help to improve treatment of obesity and other metabolic syndromes in human. The ob/ob mouse is an excellent model for obesity and diabetes (23). These mice are leptin deficient genetically, which causes excessive overeating and development of obesity, steatosis, steatohepatitis, and diabetes (18). Berardinelli-Seip congenital lipodystrophy type 2 (BSCL2) is a recessive disorder characterized by an almost complete loss of WAT, insulin resistance, and fatty liver (1, 11). BSCL2 encodes a protein, seipin, whose function is largely unknown (2, 9a, 22). Fat-specific protein 27 (Fsp27), a member of the Cide family of proteins, was shown to localize to lipid droplet and promote lipid storage in adipocytes (34). To better understand the regulation effect of ob, seipin, or Fsp27 gene on BAT and WAT, we evaluated BAT and WAT in the mouse models of ob gene mutation (ob/ob), the human BSCL2/seipin gene knockout (SKO) mice, and the Fsp27 gene knockout (Fsp27\(^{−\text{/−}}\)) mice using magnetic resonance (MR) imaging and \(^1\)H-MR spectrocope (MRS).

Compared with positron emission tomography that has been used previously for investigating BAT deposits in humans (8, 35, 36), MR approaches have better spatial resolution at lower cost and are safer, without the radioactive tracers being introduced (26). MR offers several noninvasive methods to quantify the fat fraction (FF), including single-voxel \(^1\)H-MRS, which gives a spectrum covering several detectable resonances from fat or lipid molecules, and MR imaging, which acquires separate fat and water images based on the frequencies of the protons from fat and water. Since the adipose tissue has small size, great efforts have been undertaken to minimize the voxel size for MRS measurements (30). Alternatively, MR imaging provides a large coverage of organ or tissue of interest with higher spatial resolution, which is widely used to investigate...
the fat distribution. More specifically, chemical shift-selective imaging (CSSI), an approach that combines the imaging and MR spectroscopy method, can be applied to quantify FFs of BAT in normal rats, as shown in earlier studies (20, 27).

In our previous study, we demonstrated that CSSI and $^1$H-MRS were accurate in quantifying FFs in mouse liver (25). Here, we further apply this approach to evaluate the effect of gene knockouts on FFs in the BAT and WAT using ob/ob, SKO, and Fsp27$^{-/-}$ mice and to compare these MR-derived data with those obtained from the histological analysis.

MATERIALS AND METHODS

Animals. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Medical School of Southeast University (approval ID: SYXX-2010.4987). Five ob/ob mice (average weight of 47.50 ± 1.54 g) and five wild-type (WT) mice (C57BL/6J, average weight of 26.00 ± 0.71 g) were obtained from the Laboratory Animal Center of Academy of Military Medical Science and Shanghai Animal Model Research Center, respectively. Four SKO mice (average weight of 21.80 ± 1.61 g) and four Fsp27$^{-/-}$ mice (average weight of 25.25 ± 1.55 g) were given by Peking University Health Science Center and Tsinghua University Department of Biological Sciences and Biotechnology, respectively. All animals were male and 10 wk old. They were fed a normal diet and kept at a temperature of 20–24°C before the experiments.

Protocols for MR imaging and MR spectroscopic data acquisitions. For in vivo MR experiments, anesthesia was applied to animals by inhalation of a mixture of oxygen and 5% isoflurane. Body temperature of the animal was maintained at 37 ± 0.2°C. The MR scan room temperature was kept at 22 ± 1°C. All MR experiments were performed on a 7T small animal MR system with a maximum gradient strength of 375 mT/m (Bruker PharmaScan, Ettlingen, Germany). A 38-mm inner diameter receiver/receive quadrature volume coil was used for data collection. The mouse was positioned prone inside the coil before being placed in the scanner. To minimize motion artifacts from breathing, the respiratory rate of mice were maintained at 15–20 times/min.

T$_1$-weighted images were acquired with a respiratory-gated spin echo sequence, with repetition time (TR)/echo time (TE) of 500/15 ms, section thickness of 1 mm, matrix of 256 × 256, field of view of 3.5 × 3.5 cm, four excitations, and an acquisition time of 5 min and 32 s. The T$_1$-weighted images were used to study the distribution of fat stores. The volume of the WAT was measured using Image J software (National Institutes of Health, Bethesda, MD).

Localized single-voxel $^1$H-MRS was performed using a point-resolved spectroscopy sequence without water suppression. The acquisition parameters were as follows: TR/TE, 2,500/20 ms; voxel, 1.5 × 1.5 × 1.5 mm; number of excitations, 128; and acquisition time, 5 min and 30 s. The voxels were placed precisely over BAT in the interscapular region and WAT in the retroperitoneal region, avoiding muscle or other tissue as much as possible (Fig. 1A). The phase and the baseline of the spectra were corrected with great care using TOPSPIN (Bruker PharmaScan MRI). Spectra were used only if homogeneity after shimming was better than 0.45 ppm (130 Hz), measured as the full width at 50% peak height. To correct for T$_2$ effects, seven consecutive spectra, with different echo times of 10, 20, 30, 40, 50, 70, and 90 ms, were used to measure the T$_2$ relaxation time of the selected metabolites. T$_2$ relaxation time was determined for nine different peaks (ranging from 0.9 to 5.32 ppm; Fig. 1B) from the protons of fat/lipid molecules by fitting the monoexponential model function $M_{ET} = M_0 \times \exp(-TE/T_2)$. The T$_2$ correction factors were calculated from $M_2/M_{ET}$. The spectra with T$_2$ correction were used for FF analysis. FF measured by this technique was calculated using the following formula: 

$$\text{FF}_{\text{CSSI}} = 100 \times \frac{\text{integral value of the fat peak}}{\text{integral value of the fat peak} + \text{integral value of the water peak}}$$

Fat images and water images were obtained using CSSI based on a rapid acquisition with relaxation enhancement sequence. Both the 90° and the 180° pulses were band-selective Gaussian pulses. The frequency difference between the water and fat was ~1,000 Hz on 7T. The bandwidth of the RF pulses was 700 Hz. The slice-selection gradient was on during the 90° pulse and off during the 180° pulse, which only refocuses the spins in the selected chemical shift range. We set the center frequency for water images at 0 and 1,000 Hz lower for the fat images. All CSSI images were acquired using the following parameters: TR/TE, 1,000/9.9 ms; flip angle, 180°; section thickness, 1 mm; matrix, 256 × 256; field of view, 3.5 × 3.5 cm; no. of excitations, 4; and acquisition time, 3 min and 12 s. No cardiac or respiratory gating was applied. A region of interest was drawn at the same site as the voxel used for single-voxel $^1$H-MRS (Fig. 1A). The signal intensity (SI) in the region of interest was recorded for both fat-selective images ($S_{I_{\text{fat}}}$) and water-selective images ($S_{I_{\text{water}}}$) using the ParaVision 5.0 software (Bruker PharmaScan MRI). FF measured by CSSI was computed as follows: 

$$\text{FF}_{\text{CSSI}} = 100 \times \frac{S_{I_{\text{fat}}}}{S_{I_{\text{water}}}}$$
Histopathology. Each mouse was perfused transcardially with phosphate-buffered saline followed by freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Parts of visceral WAT and BAT in the interscapular region were fixed, dehydrated, embedded, and transversely cut into 5-μm-thick sections for hematoxylin and eosin (H & E) staining. All histopathology slides were examined, and FF analysis was performed with a histological semiautomatic vacuole segmentation procedure (HIS-S) developed with the Matlab software (The MathWorks, Natick, MA) (9). The artificial areas such as blood vessels were manually excluded by a pathologist. The percent FF used by HIS-S (FFHIS-S) was calculated by following formula: FFHIS-S = area of fat/total tissue area.

To examine the difference in the adipocyte size in four groups, we performed adipocyte analysis on H & E sections. Ten to 20 adipocyte cells were selected randomly in 400 photomicrographs, and five random microscopic fields were captured in H & E-stained sections of each mouse in each group. The selected areas were measured and calculated using Image J software (National Institutes of Health). To assess the reproducibility of the assay, blinded analysis was performed on three independent triplicate experiments. Reproducibility was assessed using intraclass correlation coefficient (ICC) on absolute agreement. The ranges of ICC values were substantial (0.81, 1.0), moderate (0.61, 0.80), fair (0.41, 0.60), slight (0.11, 0.40), and virtually none (0.00, 0.10) (29). An ICC value of >0.81 represents good agreement.

Statistical analysis. All statistical analyses were performed using SPSS software (SPSS for Windows, version 11.0, 2001; SPSS, Chicago, IL). Numerical data were reported as means ± SD. For statistical comparisons, an independent-sample t-test, paired-sample t-test, and correlation test were applied. A P value of <0.05 was considered to indicate a statistically significant difference.

RESULTS

Total volume and distribution of WAT in different models. The volume of adipose deposits was measured by the T1-weighted spin echo imaging. The total volume of body WAT in ob/ob mice was significantly higher than that of WT mice (P < 0.001) (Fig. 2, A and B). In contrast, the total volume of body WAT in SKO mice was significantly lower than that of WT mice (P < 0.001). There was a clear absence of WAT in the SKO group. In addition, there was no significant difference in the volume of body WAT between Fsp27/−/− and WT mice. However, the visceral ratio of the total volume of body WAT in Fsp27/−/− mice was significantly lower than that of WT mice (P < 0.001). The subcutaneous ratio of the total volume of body WAT in Fsp27/−/− mice was significantly higher than that of WT mice (P < 0.001). The visceral ratio of the total volume of WAT in ob/ob and SKO mice was higher than that of WT mice, but the subcutaneous ratio of the total volume of WAT in ob/ob and SKO mice was lower than that of WT mice (P < 0.05; Fig. 2C). There was a correlation between FFs in BAT calculated with MR and histological methods and the volume of body WAT (CSSI: r = 0.682; MRS: r = 0.655; HIS-S: r = 0.640; P < 0.05).

Fat fractions in WAT and BAT in different models. Two different MR methods were used to measure FFs in different gene knockout mice, given their respective advantages and limitations. FFs in WAT of ob/ob mice were higher than that of WT mice, as measured by CSSI, but no significant difference was found between ob/ob and WT group based on the values obtained by 1H-MRS and histological analyses (Figs. 2A and 4, A–C). However, CSSI and single-voxel MRS showed significant differences in the FFs of WAT between SKO or Fsp27/−/− and WT mice (P < 0.05). Specifically, the FFs in WAT of SKO and Fsp27/−/− were lower than that of WT mice. The data of FFs in WAT of SKO mice were not available from a single-voxel 1H-MRS measurement because the WAT mass was significantly small and below the minimal voxel size requirement of the single-voxel MRS method.

There were significant differences in the FFs of BAT between ob/ob and WT mice (P < 0.01), with FFs in BAT of the ob/ob mice being higher than those of WT mice, as shown in Fig. 3 and Fig. 4, A–C. There was no difference between SKO and WT mice in the FFs of BAT measured by MR methods and histological analysis. The FFs in BAT of Fsp27/−/− mice measured by single-voxel 1H-MRS and histological analysis were higher than those of control mice (P < 0.001). Results obtained from CSSI measurements followed the same trend, but with large interindividual variations. FFs in WAT of WT mice measured by three methods were significantly higher than those of BAT (P < 0.001; Fig. 4, A–C). FFs in WAT of ob/ob mice were higher than those of BAT when measured by CSSI and HIS-S, but no significant difference in results was obtained by single voxel 1H-MRS. No difference between BAT and WAT in FFs was found in SKO mice. FFs in WAT of Fsp27/−/− mice measured by CSSI and 1H-MRS were higher than those of BAT. However, no difference was found between two types of adipose tissue in Fsp27/−/− mice when measured by histological analysis.

FFs of BAT and WAT measured by CSSI and the histological method were lower than those measured by single-voxel 1H-MRS (P < 0.001), but no significant difference was observed between FFCSSI and FFHIS-S (P = 0.795). There was a strong correlation between FFs calculated by histological method and two MR methods (CSSI: r = 0.671; MRS: r = 0.736; P < 0.001). In addition, results obtained from two MR methods exhibited a linear correlation (r = 0.886, P < 0.001; Fig. 4, D–F).

H & E staining revealed that white adipocytes of WT mice contained mature adipocytes, which were uniformly characterized by the presence of a large, unilocular lipid droplet. The adipocyte size of WAT in ob/ob was significantly larger than that of the WT group (P < 0.001; Figs. 2A and 5A). In contrast, the adipocyte size of WAT in SKO mice was significantly smaller than that of WT group (P < 0.001). The WAT in SKO mice consisted almost entirely of small, immature adipocytes. Most of the WAT contained brightly eosinophilic cytoplasm and a relatively small but still distinct unilocular vacuole (lipid droplet) (9d). There was no difference in white adipocyte size between Fsp−/− and WT mice (P = 0.795). However, white adipocytes of Fsp−/− mice had small and multiple lipid droplets. Histological examination of BAT from WT, SKO, and Fsp27/−/− mice indicated that they contained multilocular lipid droplets. However, the sizes of lipid droplet in BAT of SKO and Fsp27/−/− mice were significantly larger than that of WT mice. The brown adipocyte size of SKO and Fsp27/−/− mice was also larger than that of WT mice (P < 0.001). In particular, the adipocyte size of BAT in ob/ob mice was significantly larger than that of the WT group (P < 0.001; Figs. 3 and 5B). In addition, the brown adipocytes of ob/ob mice had unilocular vacuoles (lipid droplet). The white adipocyte sizes of ob/ob, Fsp−/−, and WT mice were larger than that of brown adipocytes (Figs. 2A, 3, and 5A and B).
The intraclass correlation coefficient for intraobserver measurement reproducibility was 0.999, indicating good reproducibility between the three times. The intraclass correlation coefficient for interobserver measurement reproducibility was 0.918, indicating good reproducibility between the three observers.

Linear correlations were observed between the adipocyte sizes and FFs of BAT and WAT calculated with MR and histological methods (CSSI: $r = 0.675$; MRS: $r = 0.646$; HIS-S: $r = 0.775$; $P < 0.05$; Fig. 5C).

**DISCUSSION**

In the current study, we were able to apply MR imaging and $^1$H-MRS to determine differences in volume and distribution of WAT and the FFs of BAT and WAT in ob/ob, SKO, and...
gene knockout mice. Furthermore, it was found that smaller adipocyte size leads to lower FFs of BAT and WAT (Fig. 5) in those animal models. WAT includes subcutaneous and visceral. The subcutaneous fat is found mostly underneath the skin, in which a brown adipose determination factor (Prdm16) is selectively expressed in subcutaneous white adipocytes (28). The visceral fat, also known as organ fat, is located inside the peritoneal cavity packed between the internal organs. Leptin has a great impact on metabolic activities based on the visceral or subcutaneous regions of fat distribution that contribute to the whole body weight (4, 16, 38). The total volume of body WAT and visceral fat volume in \(\text{ob/ob}\) mice were all significantly higher compared with that of WT mice due to genetic leptin deficiency. BAT has been suggested to be involved in the mechanism protecting the animal against weight gain and obesity (13, 15). The adipocyte size of WAT and BAT in \(\text{ob/ob}\) was significantly enlarged, and the BAT also contained mature adipocytes, leading to differences in WT mice measured by MR methods and histological analysis. In data collected from the selective fat proton imaging by CSSI, the BAT depots almost disappeared, and the regions where they are usually located showed a WAT-like high-intensity signal. Such a high-intensity signal is consistent with the presence of WAT-like monolobular adipocytes in the BAT. As a consequence of their leptin deficiency and a thick layer of subcutaneous WAT, the thermal sensitivity of BAT in \(\text{ob/ob}\) mice is reduced, including diet or the temperature-induced thermogenesis pathway (14).

A near absence of WAT in the SKO mice was found in this study. The FFs of WAT in SKO mice evaluated by MR imaging and histological analysis exhibited a very distinct decrease compared with that of WT mice, which was similar to that of BAT. The adipocyte size of WAT in SKO mice was significantly smaller than that of the WT group. Cui and colleagues (6, 7) demonstrated that the white adipocytes, which were characterized by the presence of a relatively small and distinct unilocular vacuole, were immature. Szymanski et al. (32) suggested that failure to form normal lipid droplets in adipocytes or adipocyte precursors is the primary cause of BSCL2 disease. It has been shown recently that \textit{seipin} is highly expressed in adipose tissue and is strongly induced during adipocyte differentiation (5, 9b, 9c, 24). Payne et al. (24) demonstrated that knocking down \textit{seipin} in C3H10T1/2 cells impaired differentiation and caused a reduction in the expression of key genes in triacylglycerol synthesis.
when FF was lipid quantification in fat/water phantoms and animal livers circulation. smaller and multiple lipid droplets and abundant blood vessel found to acquire morphology similar to that of BAT with Fsp27 deficiency results in reduction of WAT depot and the acquisition of a brown fat-like morphology in these WAT. In Fsp27 mice measured by MR methods were all lower than those of control mice. In addition, the FFs of BAT in Fsp27 mice were all higher than those of control mice. Our in vivo data showed that FF of WAT was slightly higher than that of BAT in Fsp27 deficiency mice. Histological analysis indicated that adipocytes of WAT in Fsp27 mice had small lipid multiple droplets, but the adipocyte size was not diminution. Therefore, our findings are consistent with those from Toh et al. (34) in that WAT of Fsp27 mice were found to acquire morphology similar to that of BAT with smaller and multiple lipid droplets and abundant blood vessel circulation.

Our previous study demonstrated that CSSI was accurate in lipid quantification in fat/water phantoms and animal livers when FF was <50%, and ~9% underestimation of fat occurred from 50 to 100% (25). Because the centers of excitation frequency are methylene and water proton, the degree of FF underestimation increased in higher FF. The adipose tissue FFs usually are >50%, so CSSI underestimated FFs. Recently, studies have demonstrated that in vivo 1H-MRS of adipose tissue in mice at high magnetic field strength (7.0T) allows for localized spectroscopy in voxels as small as 1.5 × 1.5 mm (30). This technique provides more spectral information of lipid/fat molecules and enables more accurate quantification using the specific resonance selected from the spectrum. However, it is still challenging to eliminate the contamination of other tissues if the WAT or BAT is little, especially in the case of SKO mice, in which WAT are nearly absent. In this case, CSSI allowed measuring of the FF and evaluation of the distribution of WAT and BAT simultaneously. We suggest that CSSI and single-voxel MRS are complementary and can be used together to evaluate FF of WAT and BAT comprehensively.

The method of lipid extraction and quantification in vitro analysis is more accurate compared with in vivo MR methods, as demonstrated in the work by Narváez-Rivas et al. (21), in which liquid chromatographic method was applied to analyze...
phospholipids in subcutaneous fat of the Iberian pig. But the invasive biopsy or euthanization of animals is not ideal for longitudinal followup studies.

It should be noted that the current study has several limitations. First, the number of mice used in the experiments was fairly small. Second, the line width of a MR spectrum may be dependent on several factors, such as homogeneity of tissue, physiological motions, and tissue environment around the sampling voxel. Those differences may be found in different animals or different scans, leading to the variation of spectral quality. Third, after short-term cold exposure, BAT activity is strengthened by inducing a metabolic program that channels lipids into BAT (3). The chemical shift between fat and water may change in low temperature. In this study, MR scan room temperature is kept at 22°C to study physiological functions of BAT in a mouse, which is a small mammal and homeotherm. In addition to fat/water imaging, other sensitive MR imaging methods can be useful in studying BAT (17). Since BAT is highly vascularized due to the need to efficiently transport the generated heat throughout an animal’s body, techniques that measure blood flow can be developed to exploit the differences in local blood tissue perfusion rates between BAT and WAT (12). Considering the potential translation of MRS approaches for clinical application, it should be noted that the performance of the CSSI approach will be less robust at lower magnetic field strengths due to less fat/water separation. The separation of fat/water peaks is too small to quantify the FFs accurately.

CONCLUSION

In conclusion, the present work has demonstrated the feasibility of noninvasively evaluating fat fractions in the BAT and WAT of different gene knockout mice using CSSI and 1H-MR spectroscopy. The differences in fat fractions in WAT and BAT measured by MR methods were the suggested different regulation effects of ob, seipin, or Fsp27 genes on developing WAT and BAT. These are associated with smaller adipocyte size with lower fat fraction in BAT and WAT as well as distribution of WAT. The results from this study suggest that MR imaging and MR spectroscopic methods can be applied to longitudinally investigate dynamic fat fraction changes in WAT and BAT due to temperature and other manipulations.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

X.-G.P. and S.J. did the conception and design of the research; X.-G.P., F.F., K.F., X.C., G.L., and P.L. performed the experiments; X.-G.P. and Y.W. analyzed the data; X.-G.P. interpreted the results of the experiments; X.-G.P. prepared the figures; X.-G.P. drafted the manuscript; S.J., H.M., and G.-J.T. edited and revised the manuscript.

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