Seipin ablation in mice results in severe generalized lipodystrophy

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Berardinelli-Seip congenital lipodystrophy type 2 (BSCL2) is an autosomal recessive disorder characterized by an almost complete loss of adipose tissue, insulin resistance and fatty liver. Here, we create the first murine model of BSCL2 by targeted disruption of seipin, the causative gene for BSCL2. Compared with their wild-type littermates, the seipin−/− mice are viable and of normal weight but display significantly reduced adipose tissue mass, hepatic steatosis, glucose intolerance and hyperinsulinemia. The levels of leptin and adiponectin were both significantly decreased in seipin−/− mice, so were non-esterified fatty acids upon fasting. Surprisingly, however, hypertriglyceridemia which is common in human BSCL, was not observed in seipin−/− mice. Our findings suggest a possible tissue-autonomous role of seipin in liver lipid storage. The availability of the seipin−/− mice should help elucidate the molecular function of seipin and lead to a better understanding of the many metabolic consequences of human BSCL2.

INTRODUCTION

Congenital generalized lipodystrophy (CGL, also known as Berardinelli-Seip congenital lipodystrophy, or BSCL), is an autosomal recessive disorder characterized by a near total loss of adipose tissue, severe insulin resistance, hypertriglyceridemia and fatty liver (1). Genome-wide linkage analysis has identified two loci for CGL: CGL type 1 (CGL1) is caused by mutations in the 1-acylglycerol-3-phosphate-O-acyl transferase 2 (AGPAT2) gene and CGL2 by mutations in the BSCL2 gene which encodes seipin (2–4). Recently, a homozygous non-sense mutation in caveolin-1 has been discovered to cause CGL (CGL3) (5). Further, mutations in polymerase I and transcript release factor (PTRF, also known as cavin) were found in five non-consanguineous patients with both generalized lipodystrophy (CGL4) and muscular dystrophy (6). PTRF/cavin is a caveolar-associated protein critical for the formation of caveolae and the stabilization of caveolins. Not surprisingly, deletion of cavin/PTRF resulted in generalized lipodystrophy (7). Both AGPAT2 and caveolin-1/cavin have clear cellular functions: AGPAT2 catalyzes the formation of phosphatidic acid (PA) and appears to control adipogenesis through modulating the synthesis of phospholipids; caveolin-1/cavin has a defined role in caveola formation. In contrast, little is known about the molecular function of seipin, despite the fact that CGL2/BSCL2 represents the most severe form of human CGLs (1).

Recent studies have examined the role of seipin in adipogenesis in cultured cells. One study demonstrated that knocking-down seipin in C3H10T1/2 cells impaired differentiation and caused a reduction in the expression of key genes in triacylglycerol (TAG) synthesis (8). Another similar study showed that knocking-down seipin in C3H10T1/2 cells did not affect bone morphogenetic protein-4-induced preadipocyte commitment. Rather, the
differentiation of pre-adipocyte 3T3-L1 cells was greatly impaired by seipin depletion, accompanied by suppression of PPARγ expression throughout the differentiation process (9). Interestingly, addition of the PPARγ agonist, pioglitazone, was able to rescue the defective differentiation caused by seipin knock-down, suggesting an intimate relationship between seipin and PPARγ.

The human BSCL2/seipin gene has three transcripts: 1.6, 1.8 and 2.2 kb as revealed by northern blot analyses: the 1.8 kb mRNA is exclusively expressed in brain and testis but the other two transcripts are ubiquitously expressed (10). Seipin is highly expressed in adipose tissue, and is strongly induced during adipocyte differentiation (8,9). However, the upregulation of seipin expression occurs only at late stages of pre-adipocyte differentiation (9). The basal level of seipin expression in the early stages of 3T3L1 differentiation appears to be critical to adipocyte development as depletion of seipin inhibits PPARγ activation at a very early stage (9).

The function of seipin in mature adipocytes remains to be elucidated, and this function may have little to do with adipogenesis. Another surprising yet exciting recent finding is that both seipin and the yeast seipin homologue, Fld1p, have been found to play a critical role in the cellular dynamics of lipid droplets (11–13). It has been suggested that Fld1p/seipin may regulate the metabolism of phospholipids/triglycerides (11,13,14). A recent study also suggests that seipin may have a structural role in the assembly of lipid droplets from the endoplasmic reticulum (ER) (15). Although progresses have been made in seipin research, the molecular function of seipin remains to be elucidated.

Despite the important role of seipin in both lipid droplet formation and adipocyte differentiation, two important aspects of human lipid storage and thereby obesity, no animal model is available to examine the in vivo function of seipin. To this end, we have generated and characterized for the first time a seipin null mouse model. Our results confirm a critical in vivo role for seipin in adipocyte development and also in hepatic lipid homeostasis.

RESULTS
Generation of the seipin null mice
We deleted the exon 3 of mouse seipin by homologous recombination using a strategy based on the Cre/loxP recombination system for generating knockout mice (Fig. 1A). Mice (C57BL/6) with loxP sites surrounding seipin exon 3 (E3fl/fl) were crossed with oocyte-specific Zp3-Cre transgenic mice to generate a seipin-null allele (seipin<sup>+/−</sup>) (Fig. 1A). Seipin<sup>−/−</sup> mice were then obtained by mating within the seipin<sup>+/−</sup> mice. The genotype was examined by PCR using the genomic DNA, and the seipin<sup>−/−</sup> mice (hereafter referred to as SKO mice) were identified with a single PCR product at 1.1 kb (Fig. 1B). The relative expression of seipin in wild-type and seipin<sup>−/−</sup> (SKO) mice was examined in various tissues by real-time (RT) quantitative PCR and by regular PCR (Fig. 1C). Finally, we detected normal expression of GNG3, a gene that is located near seipin in a head-on fashion (data not shown) (16). These results confirm that the expression of seipin but not that of GNG3 has been lost in the SKO mice.

The SKO (seipin<sup>−/−</sup>) pups can survive past weaning without unexpected early death. Mating between seipin<sup>+/−</sup> mice produced offspring with the expected 1 (seipin<sup>+/−</sup>) : 2 (seipin<sup>−/−</sup>) : 1 (seipin<sup>+/+</sup>) Mendelian ratio. Because the epididymal fat of male mice is the appropriate adipose tissue for phenotypic analyses, male mice were chosen for this initial study. In all studies, gender-, strain- and age-matched homozygous knockout animals were compared with wild-type controls. All mice were fed standard chow diet. The body weight of the SKO mice was decreased from birth until 6 weeks old but no significant difference was observed after 6 weeks (Fig. 2A). There is no difference in food consumption between wild-type and SKO mice but the SKO mice exhibited higher body temperature from time to time (Fig. 2B and C).

Plasma lipids
Total cholesterol of the SKO mice was significantly increased only at fed state while plasma glucose was significantly increased only during fasting (Fig. 2D and E). Hypertriglyceridemia, which is a common feature in human CGLs and has also been found in other lipodystrophic mouse models (17,18), was absent in the SKO mice. In fact, the level of triglyceride (TG) was dramatically decreased upon fasting (Fig. 2D and E). The level of non-esterified fatty acids (NEFA) was not changed at fed state but decreased in the SKO mice during starvation. This may likely be caused by a lack of adipose tissue in the SKO mice (see in what follows).

Dramatic loss of adipose tissue mass in the SKO mice
Previous studies found that loss-of-function mutations of seipin might be responsible for the complete loss of adipose tissue in BSCL2 patients, suggesting that seipin plays an important role in the development of adipocytes (3,4,8,9). We measured the mass of adipose depots of the SKO mice by magnetic resonance imaging (MRI) via a Bruker Pharmascan 7.0 T/16 cm spectrometer. Visual comparison of MRI images of 3-month-old wild-type and SKO mice revealed a near absence of adipose tissue in the SKO group (Fig. 3A–C). This is confirmed when little adipose was observed in the SKO mice upon dissection (Fig. 3D). The livers of the 12-week-old SKO mice were strikingly enlarged and very pale, suggesting massive deposition of fat (Fig. 3D).

The weight of individually dissected tissues and fat depots from sacrificed animals were measured (Fig. 3E and Table 1). All major fat depots were dramatically reduced in the SKO mice, where almost no gonadal fat was present (Table 1, Fig. 3E). There was also a ~60% decrease of brown adipose tissue. The liver of the SKO mice weighed almost twice as much as that of the wild-type. Although the spleen of the SKO mice also showed significant increase in weight, generalized organomegaly was not observed. The heart and kidney of the SKO mouse did not show any increase in weight (Table 1).

Histologic analyses confirm adipose tissue loss
Histologic analyses were conducted on the epididymal fat pads of 3-month-old wild-type and SKO mice. Fat pad from wild-type mice contained mature adipocytes, which were uniformly
characterized by the presence of a large, unilocular lipid droplet (Fig. 4A). In contrast, the white epididymal fat of the SKO mice consisted almost entirely of small immature adipocytes, most of which contained brightly eosinophilic cytoplasm and also a relatively small but still distinct unilocular vacuole (lipid droplet) (Fig. 4A). The subcutaneous fat of the SKO mice showed similar changes (Fig. 4B). The loss of adipose tissue in the SKO mice was further reflected by the dramatically decreased levels of plasma adiponectin and leptin, two important adipokines (Fig. 4C and D) (19,20).

Abnormal lipid accumulation in the liver of SKO mice

To understand the cause of the enlarged liver and its pale appearance in the SKO mice, we first examined liver morphology and histology (Fig. 5A and B). Fixed liver sections...
were stained with H–E and examined at different magnifications (Fig. 5B). It appears that the SKO mouse has mixed hepatic steatosis. The steatosis is mainly macrovesicular but microvesicular steatosis can also be seen. There is no obvious inflammatory cell infiltration or ballooning degeneration, suggesting no steatohepatitis at this stage. Cryosections of wild-type and SKO mouse livers were stained with oil red O, and the liver of the SKO mice contained much more lipid droplets than that of wild-type mice (Fig. 5C). Interestingly, little lipid accumulation was detected in the skeletal muscle (SkM) of the SKO mice (Fig. 5C). Finally, we measured the amount of TG in the liver and SkM of the SKO mice and wild-type littermate control. The amount of liver TG of the SKO mice was \( \approx 200\% \) higher than that of wild-type littermates (Fig. 5D). In contrast, there is little difference in SkM TG between the wild-type and SKO mice (Fig. 5D). There is no accumulation of cholesterol in the liver and SkM of the seipin mice (Fig. 5E). The increased accumulation of triglycerides in the liver could result from drastically reduced lipid storage in the adipose tissue. But a tissue autonomous role of seipin in liver lipid metabolism cannot be ruled out. The expression of some lipogenic genes such as FAS, PPAR\( \gamma \) and SCD1 was significantly increased in the SKO liver (Fig. 5F). However, the expression of SREBP-1c, the DGATs and ACC1 did not change. These data suggest that enhanced lipogenesis alone may not account for hepatic steatosis in the SKO mice. Interestingly, the expression of microsomal triglyceride transfer protein (MTP) was significantly reduced in the SKO mice, suggesting a possible defect in the lipidation of apolipoprotein B (ApoB). Given the known role for seipin in lipid droplet formation, a possible defect in the movement of neutral lipids from the cytoplasm to the lumen of the ER in the SKO liver would not be surprising.

**SKO mice is insulin resistant**

We evaluated insulin sensitivity and glucose homeostasis in the SKO and wild-type littermates at 3 months of age. Glucose tolerance test (GTT) indicated that the SKO mice showed delayed glucose clearance and were therefore diabetic (Fig. 6A). Insulin tolerance test (ITT) showed that the SKO

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**Table 1. Phenotypic comparison of wild-type and SKO mice**

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>KO</th>
<th>( P )-value</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>21.9 ± 0.9</td>
<td>21.7 ± 0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Subcutaneous fat (mg)</td>
<td>208.5 ± 15.8</td>
<td>45.8 ± 2.7</td>
<td>0.002**</td>
</tr>
<tr>
<td>Inguinal fat (mg)</td>
<td>36.6 ± 2.9</td>
<td>13.6 ± 1.7</td>
<td>0.001**</td>
</tr>
<tr>
<td>Gonadal fat (mg)</td>
<td>298.3 ± 62.6</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Retroperitoneal fat (mg)</td>
<td>87.8 ± 12.3</td>
<td>14.7 ± 2.3</td>
<td>0.004**</td>
</tr>
<tr>
<td>Mesenteric fat (mg)</td>
<td>193.5 ± 9.6</td>
<td>72.9 ± 25.3</td>
<td>0.005**</td>
</tr>
<tr>
<td>Brown adipose (mg)</td>
<td>129.6 ± 21.4</td>
<td>59.2 ± 5.9</td>
<td>0.04*</td>
</tr>
<tr>
<td>Heart (mg)</td>
<td>123.1 ± 11.2</td>
<td>126.2 ± 3.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Liver (mg)</td>
<td>1065.4 ± 67.0</td>
<td>1841.7 ± 146.4</td>
<td>0.003**</td>
</tr>
<tr>
<td>Spleen (mg)</td>
<td>82.7 ± 9.1</td>
<td>127.0 ± 10.1</td>
<td>0.02*</td>
</tr>
<tr>
<td>Kidney (mg)</td>
<td>168.4 ± 13.6</td>
<td>166.6 ± 5.3</td>
<td>0.9</td>
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Mice (12 weeks of age) were fed a standard rodent chow and sacrificed without fasting. All values are means ± s.e.m. Statistical analysis was done with two-tailed unpaired Student’s \( t \)-test. \( n = 4, \ast P < 0.05, \ast \ast P < 0.01, \ast \ast \ast P < 0.001. \)
mice had impaired insulin sensitivity compared with the wild-type littermates (Fig. 6B). They also had significantly elevated fed plasma insulin levels (Fig. 6C).

To investigate the molecular basis of the insulin resistance, the mRNA levels of insulin receptor substrate (IRS1), IRS2, Glut4 and AKT2 were examined in the liver, SkM and white adipose tissue (WAT) (Fig. 6D–F). The expression of all four genes was marked decreased in WAT. While the expression of IRS1, IRS2 and AKT2 but not GLUT4 was significantly reduced in the liver, only IRS2 expression was significantly reduced in the SkM. The expression of gluconeogenic genes such as PEPCK and G6P1 was also significantly reduced in the liver. These early results suggest that there are impaired insulin signaling in the liver, WAT and possibly the muscle of the SKO mice.

**DISCUSSION**

Recent studies have revealed important roles of seipin/BSCL2 in lipid storage at both the cellular level (lipid droplets) and the whole-body level (adipose tissue development) (1,11,12).

To better understand the in vivo function of seipin, we generated the first murine model for seipin research: the seipin<sup>−/−</sup> mouse or the SKO mice. We found that the SKO mice recapitulated many aspects of human BSLC2, such as a dramatic loss of adipose tissue, insulin resistance and hepatic steatosis. The availability of the SKO mice should lead to additional insights into the cause of metabolic disorders, e.g. insulin resistance, and help elucidate the molecular function of the mysterious yet important protein: seipin.

Both CGL1/BSCL1 (AGPAT2) and CGL2/BSCL2 (seipin) patients suffer severe fat loss. When compared with other CGL patients, CGL2/BSCL2 patients have even more severe lipodystrophy because there is a loss of both mechanical adipose tissue (found in retro-orbital, palm, sole and other areas) and metabolically active adipose tissue (found in subcutaneous, intra-abdominal, intrathoracic and other areas) (1). Interestingly, the SKO mice which clearly suffer from severe lipodystrophy, still retain a significant portion of both WAT and BAT (Table 1), whereas the AGPAT2-deficient mice has an almost complete loss of white and brown adipose tissues (21). Although not 100% identical, the genetic backgrounds of the mice used in these two studies are very similar. These data suggest that AGPAT2, but not seipin, might play a more important role in adipocyte development in mice.

Hypertriglyceridemia and hepatic steatosis are common features of human CGL. Not surprisingly, both AGPAT2-deficient and SKO mice develop severe hepatic steatosis. However, chronic steatohepatitis was observed in the AGPAT2 mice but not in the SKO mice (21). In the case of hypertriglyceridemia, although similar rates of CGL1 and CGL2 patients (62.5% for CGL2/seipin patients and 71% for CGL1/AGPAT2) develop hypertriglyceridemia, there is a major difference between the two mouse models (22). In the SKO male mice, the plasma TG level is not elevated. In fact, the level of plasma TG dropped by over 80% upon

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Figure 4. Impaired adipocyte development of the SKO mice. (A, B) Histological section (H&E) of epididymal (A) and subcutaneous (B) WAT from 3-month-old SKO and control mice under chow diet. Bars, 400 μm (top) and 40 μm (bottom). Serum adiponectin (C) and leptin (D) levels of WT and SKO mice (SKO n = 7, WT n = 10, *P < 0.05, ***P < 0.001).
The AGPAT2-deficient male mice, on the other hand, developed hypertriglyceridemia when fed with standard chow without fasting. This may be explained in part by the severity of hepatic steatosis: the TG of a 3-month-old SKO liver increased by \( \approx 200\% \), whereas the TG of a similar aged AGPAT2-deficient liver by \( \approx 500\% \). There was also highly elevated hepatic lipogenesis in the AGPAT2-deficient mice, whereas only a moderate increase in the expression of FAS, SCD-1 and PPAR\( \gamma \) was detected in the SKO mice. In fact, the expression level of ACC1, SREBP-1c and the DGATs did not change in the SKO mice (Fig. 5F). Although other possibilities cannot be ruled out, these differences imply that seipin and AGPAT2 may function through different mechanisms to regulate hepatic lipid homeostasis.

The decrease in plasma TG of the SKO mice upon fasting may be caused by reduced lipidation of nascent ApoB, as the expression of MTP was significantly reduced (Fig. 5). MTP plays an essential role in transferring the bulk of triglycerides into the lumen of the ER for very low density lipoprotein (VLDL) assembly and is required for the secretion of ApoB-100 from the liver (23). Therefore, a reduction in MTP expression may be partially responsible for the decrease of plasma TG upon fasting. There is also an intimate relationship between cytoplasmic lipid droplets and the maturation of VLDL, and a recent study demonstrated that the ER- and lipid-droplet-associated protein CideB mediates VLDL lipidation and maturation (24). Given its known role in lipid droplet formation and its localization to the ER and lipid droplets, seipin could take part in the movement of the neutral lipids across the ER and thereby directly or indirectly regulating the lipidation of nascent ApoB.

Similar to the AGPAT2 deficient mice but different from all other mouse models of lipodystrophy associated with hepatic steatosis, the level of NEFA was normal or low (upon fasting) in the SKO mice (21,25,26). The lack of adipose tissue is probably a major contributing factor. Likewise, VLDL may not be efficiently secreted from the liver in the absence of seipin, resulting in decreased plasma NEFA upon fasting. This again suggests that seipin may have a tissue autonomous function in lipid storage and secretion in the liver. Importantly, these results also suggest that high serum NEFA levels are not required for the development of hepatic or peripheral insulin resistance, in agreement with an early study on AGPAT2 (21).

In summary, we have created the first mouse model in seipin and BSCL2 research and conducted initial

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**Figure 5.** Hepatic steatosis in the SKO mice. (A) Photograph of the liver of WT (left) and SKO (right) mice. (B) Histological analysis (hematoxylin/eosin staining) of liver sections with different magnification from SKO (bottom right) mice filled with vacuoles (black arrow), compared with WT littermates (bottom left). Bars, 400 \( \mu \)m (top) and 40 \( \mu \)m (bottom). (C) Images of liver and skeletal muscle (gastrocnemius) sections stained with oil red O, the red color droplets represent the lipid droplets in the liver of SKO mouse (scale bar is 50 \( \mu \)m). Liver and skeletal muscle TAG (D) and TC (E) contents from 3-month-old SKO mice and WT littermates (\( n = 4, **P < 0.01 \)). (F) Expression of genes concerned with lipid synthesis and transportation in the livers of SKO mice and control littermates (\( n = 4, *P < 0.05, **P < 0.01 \)). ACC1, acetyl CoA carboxylase 1; DGAT, diacylglycerol acyl-transferase; FAS, fatty acid synthase; MTP, microsomal transfer protein; PGC-1alpha, PPAR gamma coactivator 1alpha; PPARg, peroxisome proliferator-activated receptor gamma; SCD-1, stearoyl-coenzyme A desaturase 1; SREBP1c, sterol response element binding protein 1c.
characterization. Although much is to be done, our findings clearly demonstrated a crucial role of seipin in adipocyte development in mice. Future exploitation of this unique in vivo model should lead to exciting new developments in seipin, lipodystrophy and metabolic research.

MATERIALS AND METHODS

Animals

Two loxP sites and a neo cassette flanked by two FRT recombination sites in seipin intron 2 and 3, respectively, were introduced into the seipin locus by homologous recombination in ES cells (Fig. 1A). The targeting construct was electroporated into 129 ES cells and G418 resistant clones were selected under standard conditions. Appropriately targeted ES cells were identified by PCR and Southern blotting and were then injected into C57BL/6 blastocysts. The blastocysts were implanted into pseudopregnant females. Male chimaeras positive for germ-line transmission were used to establish recombinant mouse lines. The neo gene was excised through breeding with Flp recombinase-expressing mice (JAX Strain #003946). Germ-line deletion of seipin exon 3 was induced by crossing mice with the loxP seipin allele to transgenic mice expressing Cre recombinase driven by a germ (oocyte) cell specific promoter (Zp3-cre, Jax #003394) (27). Progeny were screened by PCR for loss of the seipin exon 3. After the generation of the seipin-null allele (seipin+/-), the strain was further crossed with C57BL/6 for three generations and inbred with heterozygotes to obtain homozygotes.

The genotyping was examined by PCR using the genomic DNA obtained from the clipped tail. Primers used for the seipin gene were Seipin-1 (5'-TCTATGGCTTCTTCTACTC-3'), Seipin-2 (5'-ACTAAAGGCAAAGGAGG-3'), and for the wild-type allele were Seipin-1 (5'-TCTATGGCTTCTTCTACTC-3'), Seipin-2 (5'-ACTAAAGGCAAAGGAGG-3'). Using a mixture of these primers, PCR was performed with 35 cycles of a reaction consisting of 30 s of denaturation at 94°C, 30 s of annealing at 56°C and 1 min of elongation at 72°C. PCR products were 1100 and 300 bp, specific for null and wild alleles, respectively.

All experiments involving mice were approved by the Institutional Animal Care Research Advisory Committee of the National Institute of Biological Science and Animal Care Committee of Peking University Health Science Center. Animals were housed and allowed free access to tap water and standard laboratory chow. The ‘Principles of Laboratory Animal Care’ (NIH publication no. 85–23, revised 1996) were followed.

All mice were maintained on a 12 h light/dark cycle and were fed ad libitum with regular mouse chow (4% fat by weight). Mice were fasted for 4 h and blood samples were taken from the retro-orbital plexus. The liver, heart, kidney, brain and SkM were then harvested, snap-frozen in liquid nitrogen and stored at −80°C for real-time PCR.

RNA isolation and quantitative real-time PCR

Total RNA was extracted using Trizol reagent (Invitrogen, USA) and first-strand cDNA was generated by using a RT kit (Invitrogen, USA). Quantitative real-time PCR was performed using primer sets shown in Table 2.

Amplifications were performed in 35 cycles using an opticon continuous fluorescence detection system (MJ Research) with SYBR green fluorescence (Molecular Probes, Eugene, USA). Each cycle consisted of heating denaturation for 30 s at 94°C, annealing for 30 s at 56°C and extension for 30 s at 72°C. All samples were quantitated by using the comparative CT method for relative quantitation of gene expression, normalized to GAPDH (28).

Figure 6. SKO mice are insulin resistant. Glucose (A) and insulin (B) tolerance tests performed on 3-month-old male mice on regular diet (n = 4, *P < 0.05, **P < 0.01, ***P < 0.001). (C) Increased serum insulin level in SKO mice compared with control littermates (SKO n = 7, WT n = 10, **P < 0.01). Expression of selected genes in the liver (D), skeletal muscle (E) and WAT (F) (n = 4, *P < 0.05, **P < 0.01, ***P < 0.001).

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Blood analysis

Blood was obtained by retro-orbital bleed. Plasma TC, TG and glucose were determined by using enzymatic methods (Sigma kits, MO, USA). Serum insulin, leptin and adiponectin were measured by ELISA (Linco Research, St Charles, MO), and free fatty acids were measured by using a free fatty acid kit (Wako).

Glucose and insulin tolerance tests

For glucose and insulin tolerance tests, mice fasted for 4 h were given i.p. glucose (2 g/kg body weight; Abbott) or insulin (Humulin, 0.75 mIU/g), respectively, and blood samples were collected before (time 0) and at 15, 30, and 60 and 120 (90 for ITT) min after injection for measurement of glucose (Sigma kits, MO, USA).

Histological studies

Tissues were fixed in 4% neutral formalin and paraffin-embedded, and sections were stained with hematoxylin/eosin. The segments of liver and SkM were cryostat sectioned at a thickness of 7 μm onto poly-l-lysine slides for lipid deposition analysis by oil red O staining. Adipose tissue was prepared and subjected to hematoxylin and eosin staining. The wild-type littermates were used as controls.

Analysis of liver lipids

Approximately 100 mg of liver (wet weight) was weighed and homogenized in 1 ml PBS. Lipids were extracted as described by Folch et al. (29) and dissolved in 100 μl 3% Triton X-100. The determination of TG was carried out using enzymatic methods as described earlier.

Magnetic resonance imaging

For MRI acquisition, anesthesia was induced by inhalation of a mixture of oxygen and 5% isoflurane and maintained by a mixture of oxygen containing 1–2% isoflurane. Mice were positioned and immobilized prone inside the tomograph with either the thoracic or the abdominal region in the center of the field of view (FOV). All MRI experiments were carried out using a 7 T small animal magnetic resonance tomograph with Bruker Pharmascan 7.0 T/16 cm spectrometer equipped with a mini imaging gradient coil system (gradient strength, 375 mT/m) and a 1H transmit–receive quadrature coil with 31 mm inner diameter. T1-weighted (T1W) images were acquired with a respiratory-gated spin echo sequence, FOV 3.5 × 3.5 cm, matrix size 256 × 256, slice thickness 2.0 mm, repetition time 500 ms and echo time 15 ms and a number of repetitions (NEX) of 4. The T1W images were used to study the distribution of fat stores.

Core body temperature

Core body temperature was measured using a rectal probe (Thermalet TH-5) inserted 1.0 cm deep at ambient room temperature. Food and water were provided ad libitum.

Statistical analysis

All data are presented as means ± SEM. Statistical comparison between the two groups was performed using Student’s t-test or one-way ANOVA. A value of P < 0.05 was considered statistically significant.

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Conflict of Interest statement. None declared.

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REFERENCES