Biochemical and Biophysical Research Communications 460 (2015) 143–150

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Expression of seipin in adipose tissue rescues lipodystrophy, hepatic steatosis and insulin resistance in seipin null mice

Mingming Gao [a], Mengyu Wang [a], Xin Guo [a], Xu Qiu [a], Lu Liu [a], Jiawei Liao [a], Jinjiao Liu [a], Guotao Lu [b], Yuhui Wang [a], George Liu [a, *]

[a] Institute of Cardiovascular Sciences and Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education, Peking University Health Science Center, Beijing, 100191, China
[b] Department of General Surgery, Jinling Hospital, Medical School of Nanjing University, Nanjing, Jiangsu Province, 210093, China

ARTICLE INFO

Article history:
Received 13 February 2015
Available online 7 March 2015

Keywords:
Seipin
Global knockout mice
Adipose-specific reconstitute mice

ABSTRACT

Objectives: Gene mutations in an ER protein seipin result in congenital generalized lipodystrophy (CGL) in humans, accompanied with hepatic steatosis and insulin resistance. Seipin gene is highly expressed in the brain, testis and adipose tissue. Seipin globally deficient mice (SKO) displayed similar phenotypes as human counterparts. It has been demonstrated that adipose-specific seipin knockout mice at elder age were indistinguishable from SKO mice. Due to the large mass of adipose tissue in the body, we hypothesized that seipin in adipose tissue might be responsible for the multiple metabolism-related abnormalities in SKO mice.

Methods and Results: Transgenic mice with adipose-specific expression of human seipin gene driven by aP2 promoter were generated and crossed with SKO mice to obtain adipose-specific seipin reconstitute (Seipin-RE) mice. In comparison with wild-type (WT) and SKO mice, the Seipin-RE mice exhibited normal plasma triglyceride and non-esterified fatty acids upon fasting, recovered adipose tissue mass, restored epididymal and subcutaneous fat pads morphology and partially recovered plasma leptin and adiponectin levels. Moreover, hepatic steatosis and insulin resistance was also absent in these mice.

Conclusion: Our study demonstrates that expression of seipin in adipose tissue alone could rescue dyslipidemia, lipodystrophy, hepatic steatosis and insulin resistance in SKO mice.

1. Introduction

The primary function of adipose tissue are to store energy in the form of triglycerides during energy excess and to release energy during fasting or starvation as free fatty acids and glycerol. Adipose tissue also secretes a variety of adipokines (eg, leptin, adiponectin, TNF-α, resistin, visfatin) that play important roles in the regulation of whole body energy homeostasis and metabolism [1]. Dysfunction of adipose tissue results in obesity (adipose tissue in excess) and lipodystrophy (adipose tissue lacking). Both of these two oppose conditions share many similar complications, such as dyslipidemia, hepatic steatosis, insulin resistance and cardiovascular diseases [2].

Seipin is the causative gene of Berardinelli-Seip congenital lipodystrophy type 2 (BSCL2), one of the most severe lipodystrophy in humans, which is characterized by a nearly total loss of adipose tissue, severe insulin resistance and hepatic steatosis. Seipin encodes an integral membrane protein of the endoplasmic reticulum (ER), highly expressed in the brain, testis, and adipose tissue [3]. Dysfunctional mutations of seipin in the brain has been linked to motor neuropathy and Silver syndrome [4] while loss of seipin in mice neurons results in anxiety and depression [5]. It has been shown recently that loss of seipin in testis causes teratozoospermia syndrome in humans and in mice [6]. In consideration of adipocyte, seipin is strongly induced during adipocyte differentiation [7,8] and appears to be critical for adipocyte development. Seipin can also regulate cAMP/PKA-mediated lipolysis in adipose differentiation and essential for terminal adipocyte differentiation [9]. The function of seipin in mature adipocytes may have little to do with adipogenesis, but have important role in lipolysis [10,11]. It has been suggested that seipin may regulate the metabolism of

* Corresponding author. 38 Xueyuan Road, Haidian District, Beijing, 100191, China. Fax: +86 10 82802769.
E-mail address: georgeliu@bjmu.edu.cn (G. Liu).

http://dx.doi.org/10.1016/j.bbrc.2015.02.147
0006-291X/

phospholipids/triglycerides [12], and play a critical role in the cellular dymamics of lipid droplets [12,13]. Seipin may also have a structural role in the assembly of lipid droplets from the ER [13]. A recent study showed that seipin physically interacts with the sarco/ER $Ca2^{+}$-ATPase (SERCA) in both drosophila and humans which reveals that seipin may regulate intracellular calcium homeostasis [14].

We have generated and characterized a seipin null mouse model and confirmed a critical in vivo role for seipin in adipocyte development and hepatic lipid homeostasis. SKO mice display significantly reduced adipose tissue mass, hepatic steatosis and insulin resistance [15]. Considering seipin is highly expressed in adipose tissue, we have also generated adipose-specific seipin knockout mice, these mice have progressive lipodystrophy, and 6-month old developed hepatic steatosis and insulin resistance [11].

We hypothesized that seipin in adipose tissue plays a critical role for the multiple metabolism-related phenotypes in SKO mice. To confirm this, we expressed seipin in adipose tissue of SKO background, then studied the change of phenotypes and clarified the important role of seipin in adipose tissue. Our results reveal that expression of seipin in adipose tissue could rescue dyslipidemia, lipodystrophy, hepatic steatosis and insulin resistance in SKO mice, which provides clear evidence that seipin in adipose tissue is responsible for the multiple metabolic associated abnormalities.

2. Materials and methods

2.1. Animals

All mice were maintained on a 12-h light/dark cycle and were fed ad libitum with regular chow diet. All experiments involving mice were approved by the Animal Care Committee of Peking University Health Science Center. The 'Principles of Laboratory

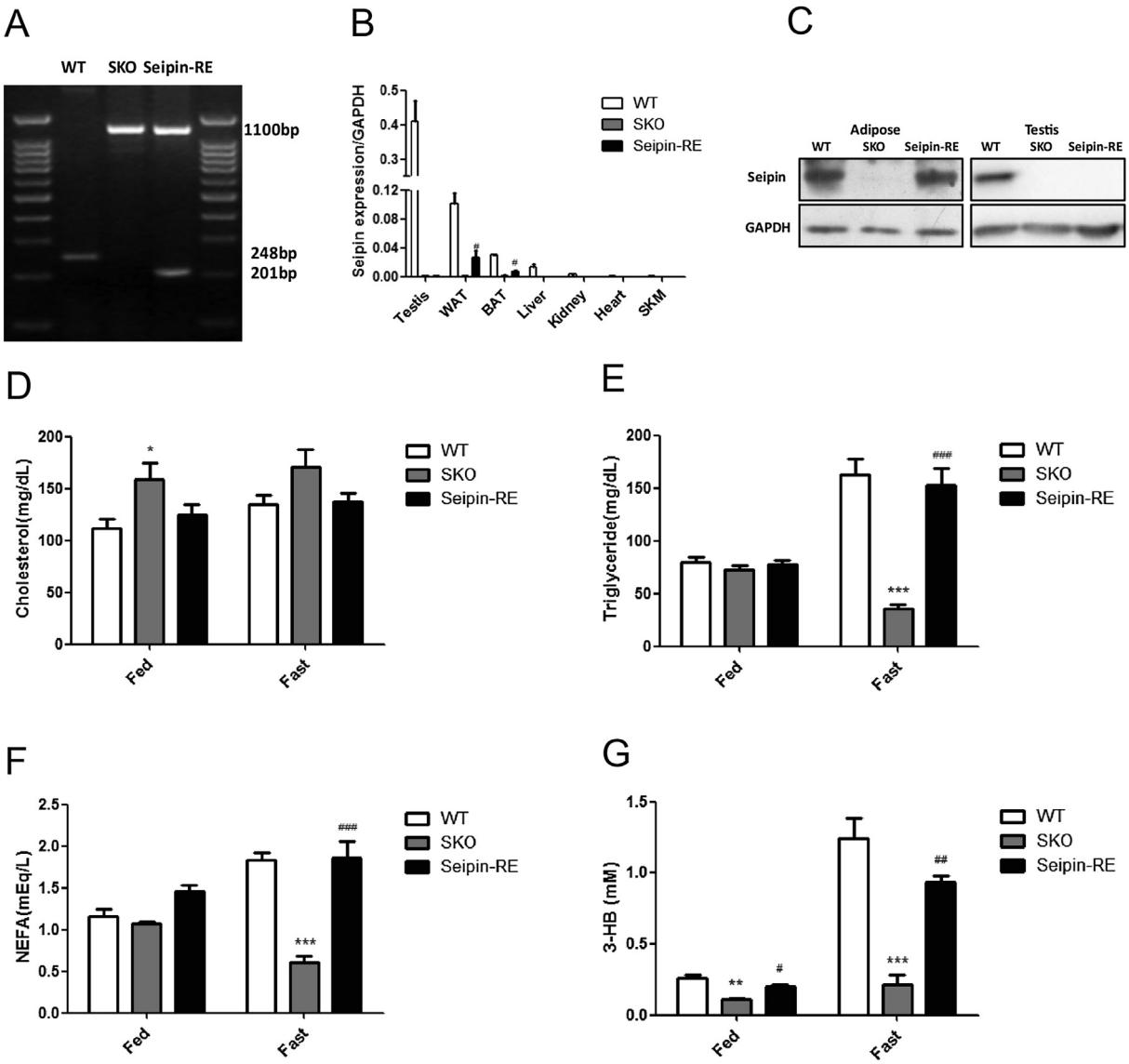


Fig. 1. Generation and characterization of Seipin-RE mice. (A) Genotyping PCR of tail clips of WT (lane 2), SKO (lane 3), and Seipin-RE (lane 4) mice. 248-bp product from WT seipin locus, 1100-bp product from deleted seipin locus and 201-bp from human seipin transgenic locus. (B) mRNA expression of seipin in different tissues by real time PCR. White adipose tissue (WAT), brown adipose tissue (BAT), skeletal muscle (SkM) (n = 5). (C) Seipin protein expression in WAT and testis by western bloting. Fed and 16-h fasted states plasma total cholesterol (D), triglyceride (E), NEFA (F) and β-hydroxybutyrate (3-HB) (G) levels in 3-month old WT, SKO and Seipin-RE mice (n = 5–8). (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with WT mice; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ compared with SKO mice).

Animal Care' (NIH publication No. 85-23, revised 1996) were followed.

Adipose-specific seipin transgenic mice carrying a short isoform of human seipin gene (encoding 398 amino acids) using the adipose-specific aP2 promoter were generated [10], and crossed with SKO mice [15], to obtain adipose-specific seipin reconstitute (Seipin-RE) mice. These mice were in C57BL/6J background. WT, SKO and Seipin-RE mice used in this study were littermates and with half males and half females. The genotyping was examined by PCR using the genomic DNA obtained from the clipped tail. Primers used for the seipin knockout mice were Seipin-1 (5′-TCTAT GGCTCCTTCTACTACTC-3′), Seipin-2 (5′-CGAATGATATGACGAC GACT-3′) to product mutant allele, and for the wild type allele were Loxp-F (5′-CTTGTCTCAAAGGGGTCT-3′), Loxp-R (5′-TCAACAGAA-CAGACGCT-3′). Primers used for the adipose-specific seipin transgenic mice were hSeipin-F (5′-TATGCGGCCGCTCAGGAACTAGAG CAGG-3′) and hSeipin-R (5′-TATGATATCATGGTCAACGACCCTCC-3′). PCR products were 1100 bp, 248 bp, and 201 bp, specific for null allele, wild type allele and transgenic allele, respectively.

2.2. RNA isolation and quantitative real-time PCR

Total RNA was extracted using Trizol reagent (Invitrogen, USA) and first-strand cDNA was generated by using an RT kit (Invitrogen, USA). Quantitative real-time PCR was performed using primers shown in Supplementary Table S2. Amplifications were performed using an opticon continuous fluorescence detection system (MJ Research) with SYBR green fluorescence (Molecular Probes, Eugene, USA). All samples were quantitated by using the comparative CT method for relative quantitation of gene expression, normalized to GAPDH [16].

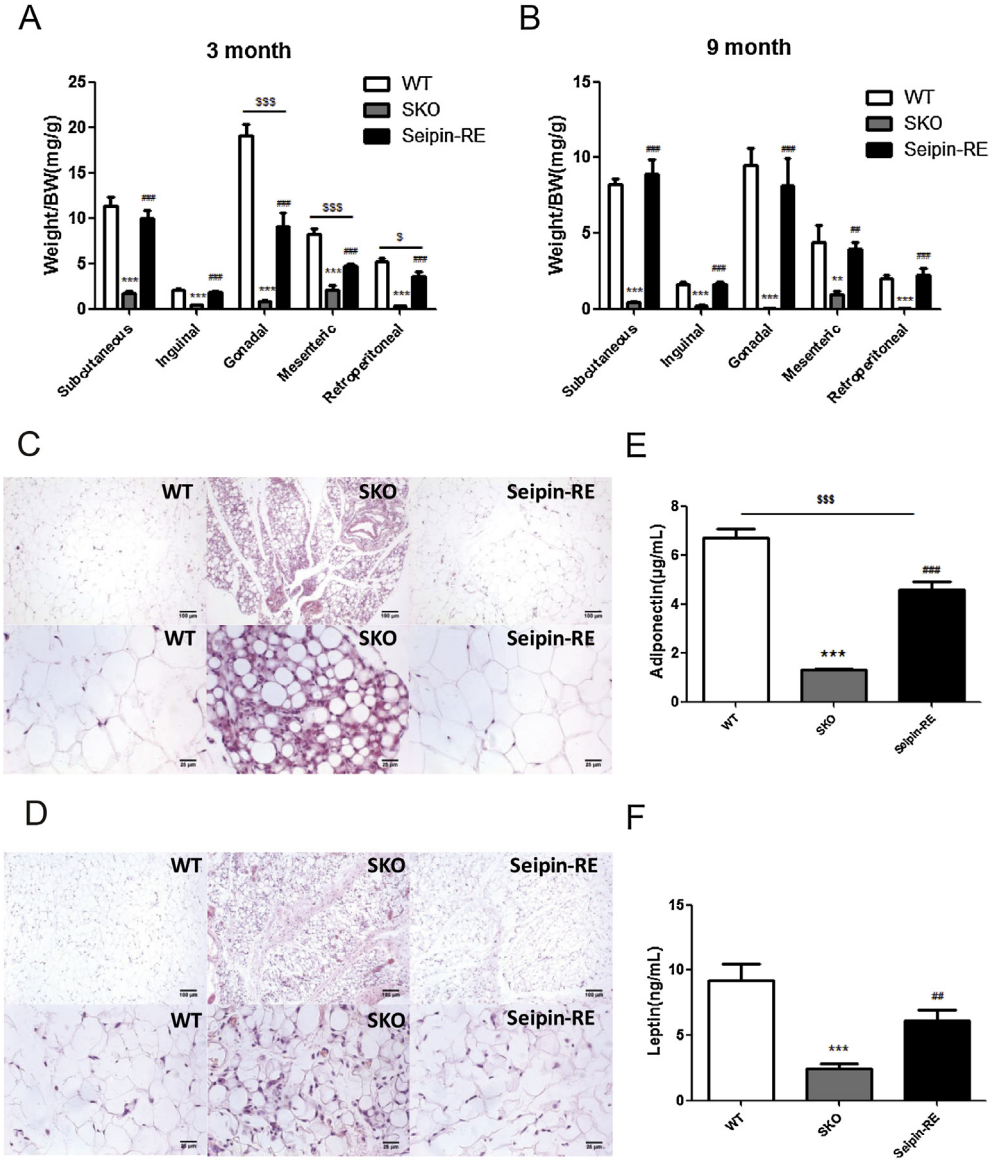
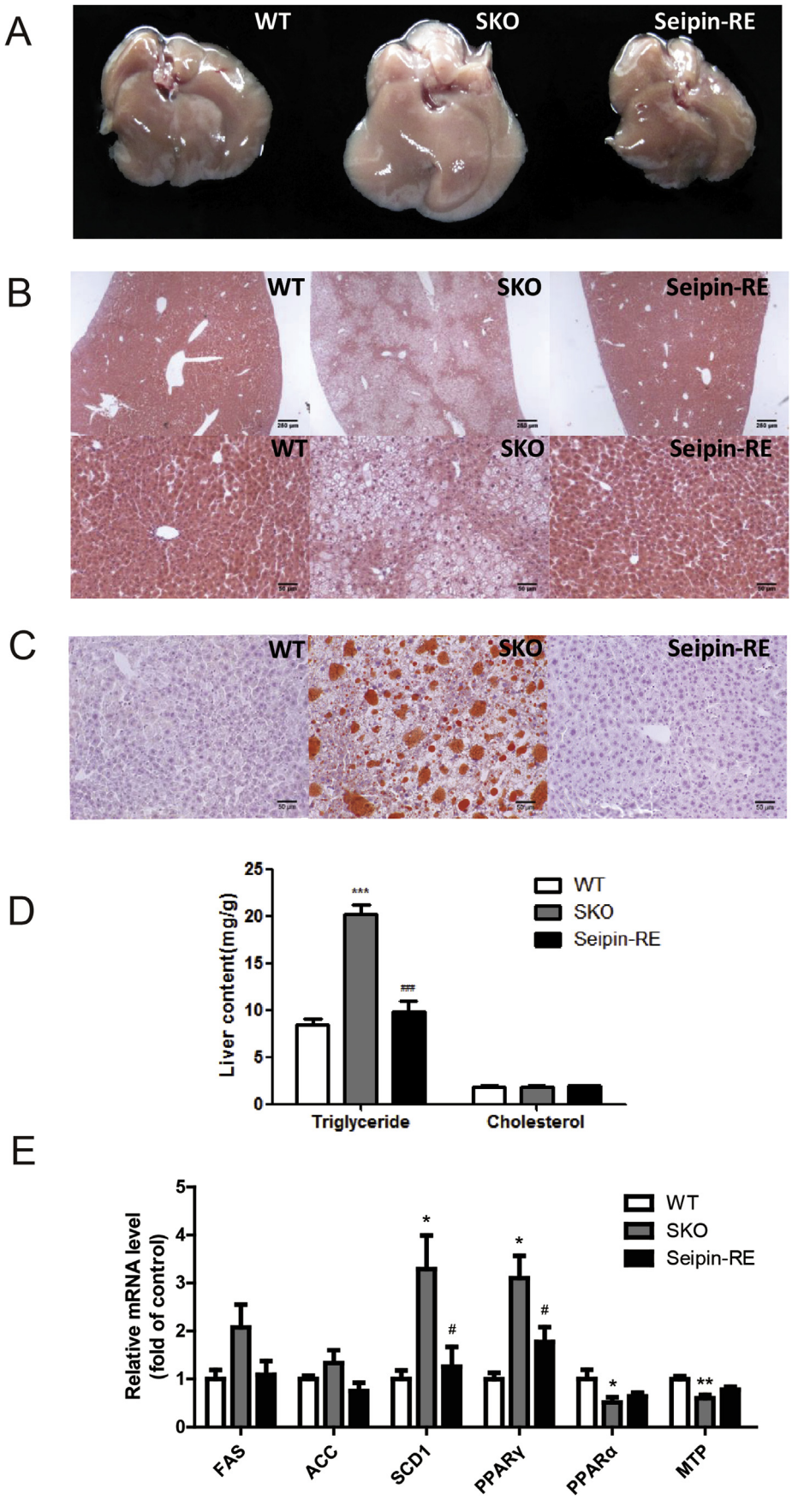


Fig. 2. Lipodystrophy was recovered in Seipin-RE mice. The major fat depots of WT, SKO and Seipin-RE mice were weighed when sacrificed. The reduced adipose tissue mass in SKO mice was partially (63% of wild type mice) and completely recovered in 3-month (A) and 9-month (B) old Seipin-RE mice. Histological section (H&E staining) of epididymal (C) and subcutaneous (D) fat pads from 3-month old WT (left), SKO (middle) and Seipin-RE (right) mice under chow diet. Bars, 100 μm (top) and 25 μm (bottom). Serum adiponectin (E) and leptin (F) levels of 3-month old WT, SKO and Seipin-RE mice. (n = 8, $^{***}p < 0.001$ compared with WT mice; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with SKO mice; $^{\$}p < 0.05$, $^{\$\$\$}p < 0.001$ compared with WT mice).


A
WT
SKO
Seipin-RE
B
WT
SKO
Seipin-RE
WT
SKO
Seipin-RE
C
WT
SKO
Seipin-RE
D
Liver content(mg/g)
25
20
15
10
5
0
***
###
WT
SKO
Seipin-RE
Triglyceride
Cholesterol
E
Relative mRNA level
(fold of control)
5
4
3
2
1
0
*
#
*
#
*
**
WT
SKO
Seipin-RE
FAS
ACC
SCD1
PPARγ
PPARα
MTP

2.3. Western blot analysis

White adipose tissue and testis were homogenized in RIPA buffer containing complete protease inhibitor cocktail tablets (Roche, USA). The protein content of tissue lysates was determined using a bicinchoninic acid protein assay kit (Pierce, USA). Tissue lysates were subjected to western blotting. A rabbit polyclonal antibody against seipin was used as previously described [6].

2.4. Analysis of blood lipids, glucose, insulin, leptin, adiponectin and β-hydroxybutyrate

Blood was obtained by retro-orbital bleeding. Plasma total cholesterol (TC), triglyceride (TG) and glucose were determined by enzymatic methods (Sigma kits, USA), and non-esterified fatty acids (NEFA) were measured by using an NEFA kit (Wako, Japan). Serum insulin, leptin, and adiponectin were measured by ELISA (Linco Research,USA). Plasma β-hydroxybutyrate was measured by using a colorimetric assay kit (Cayman Chemical Company, USA).

2.5. Histological studies

Adipose tissue and liver were fixed in 4% paraformaldehyde, paraffin-embedded, and sections were stained with hematoxylin/eosin. Fixed liver was embedded in OCT (Sakura Finetek, USA) and cryostat sectioned at a thickness of 7 μm onto poly-L-lysine slides for lipid deposition analysis by oil red O staining.

2.6. 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. [17] and dissolved in 500 μl 3% Triton X-100. The determination of triglyceride and cholesterol were carried out using enzymatic methods as described above.

2.7. 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; Sigma, USA) or insulin (Humulin, 0.75 IU/kg body weight, Lilly, Feancy), respectively, and blood samples were collected before (time 0) and at 15, 30, 60 and 120 (for GTT) or 90 (for ITT) min after injection for measurement of glucose (Sigma kits, USA).

2.8. Statistical analysis

All data were presented as means ± SEM. Statistical was performed using one-way ANOVA, followed by the Turkey posttest for comparison among groups using GraphPad Prism 5.0. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Seipin-RE mice were generated

To assess the effects of seipin in adipose tissue, SKO mice were crossed with adipose-specific seipin transgenic mice to obtain Seipin-RE mice. These mice expressed seipin in adipose tissue on a seipin deficient background. The genotyping was examined by PCR using the genomic DNA. Seipin-RE mice were identified with two PCR products at 1100 bp and 201 bp (Fig. 1A). The relative mRNA expression of seipin in WT, SKO and Seipin-RE mice was examined in various tissues by real-time quantitative PCR (Fig. 1B). Seipin mRNA in the testis, liver, heart, kidney, and skeletal muscle from Seipin-RE mice was undetectable but was 30% or 23% of WT mice in white and brown adipose tissue respectively. By western bloting using a rabbit antibody specific for mouse seipin, a protein band at size of 55 kd was detected in white adipose tissue (WAT) of WT and Seipin-RE mice but was absent in SKO mice. A band between 55 kd and 70 kd was present in testis of WT but absent in both SKO and Seipin-RE (Fig. 1C). The observed size difference of seipin protein from adipose tissue and testis maybe because of different post-translational modifications in different tissues. Only 30% recovered mRNA in adipose tissue was enough to translate WT levels of seipin protein in Seipin-RE mice. Therefore, Seipin-RE mice expressed seipin specifically in adipose tissue.

3.2. Fasting induced hypolipidemia was restored in Seipin-RE mice

We measured plasma lipid and β-hydroxybutyrate (3-HB) concentrations upon fed and 16-h fasted states of 3-month old mice. The cholesterol level was slightly increased upon fed state in SKO mice and recovered in Seipin-RE mice (Fig. 1D). Although hypertriglyceridemia is a common feature of BSCL2 patients, SKO mice displayed normal plasma triglyceride and NEFA in fed state, but dramatically decreased upon fasting, while Seipin-RE mice were completely restored (Fig. 1E and F). 3-HB, a ketone, is produced by the liver from free fatty acids. We found significantly reduced plasma 3-HB level in fed and fasted states of SKO mice, while Seipin-RE mice were normally compared with WT mice (Fig. 1G). WT and Seipin-RE mice could increase their 3-HB level upon fasting, however SKO mice were unable to do this (Fig. 1G). Therefore, Seipin-RE mice restored fasting induced hypolipidemia in SKO mice.

3.3. Lipodystrophy was recovered in Seipin-RE mice

The weight of individually dissected tissues and fat depots from 3-month old sacrificed animals were measured (Supplementary Table S1). All major fat depots were dramatically reduced in the SKO mice. The reduced adipose tissue mass was partially (63% of WT mice) and completely recovered in the 3-month (Fig. 2A and Table S1) and 9-month (Fig. 2B) old Seipin-RE mice. Histologic analyses were conducted on the epididymal and subcutaneous fat pads of 3-month old mice. Fat pads from SKO mice consisted almost entirely of small immature adipocytes, while Seipin-RE mice contained mature adipocytes, which were uniformly characterized by the presence of a large, unilocular lipid droplet the same as WT

Fig. 3. Hepatic steatosis was rescued in Seipin-RE mice. (A) Photograph of the liver of 3-month old WT (left), SKO (middle) and Seipin-RE (right) mice. (B) Histological analysis (H&E staining) of liver sections with different magnification from 3-month old WT (left), SKO (middle) and Seipin-RE (right) mice. Bars, 250 μm (top) and 50 μm (bottom). (C) Images of liver sections stained with oil red O from 3-month old WT (left), SKO (middle) and Seipin-RE (right), the red color droplets represent the lipid droplets (scale bar is 50 μm). (D) The lipid contents of liver from 3-month old WT, SKO and Seipin-RE mice ($n = 8$). (E) Expression of genes concerned with lipid synthesis and transportation in the livers of 3-month old WT, SKO and Seipin-RE mice ($n = 8$). ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with WT mice; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ compared with SKO mice). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

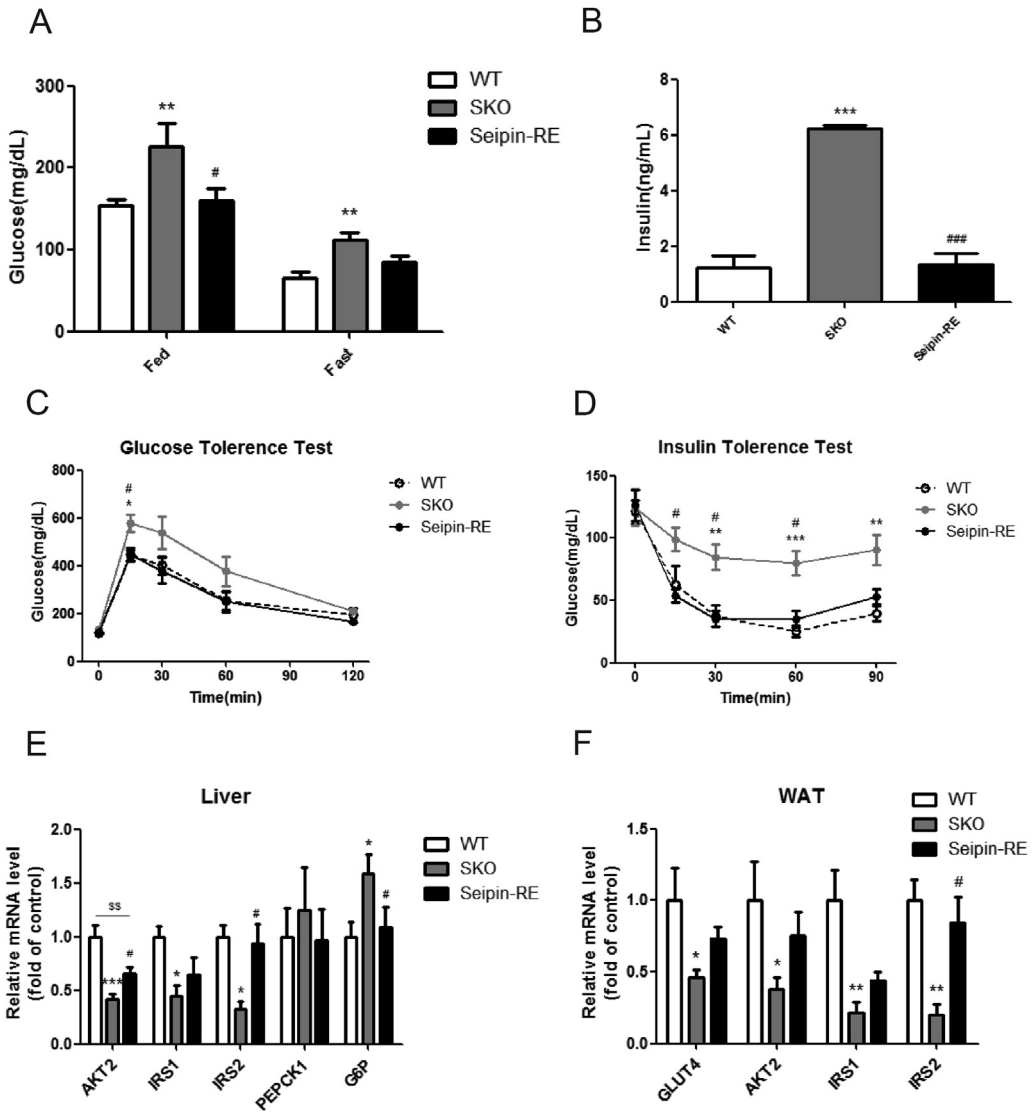


Fig. 4. Insulin resistance was normalized in Seipin-RE mice. (A) Fed and 16-h fasted states plasma glucose levels in 3-month old WT, SKO and Seipin-RE mice. (B) Fed state plasma insulin levels of 3-month old WT, SKO and Seipin-RE mice (n = 8). Glucose (C) and insulin (D) tolerance tests performed on 3-month old mice on chow diet upon 4-h fasting (n = 5–6). Expression of insulin signaling associated genes in the liver (E) and WAT (F) of 3-month old mice (Liver n = 8, WAT n = 7). (*p < 0.05, **p < 0.01, ***p < 0.001 compared with WT mice; #p < 0.05, ###p < 0.001 compared with SKO mice; $$p < 0.01 compared with WT mice).

mice (Fig. 2C and D). The decreased levels of plasma adiponectin and leptin, two important adipokines in the SKO mice were also rescued in Seipin-RE mice (Fig. 2E and F).

3.4. Hepatic steatosis was rescued in Seipin-RE mice

We detected the severity of hepatic steatosis in 3-month old mice. The liver of SKO mice was enlarged and appearance pale upon dissection and liver weighted (Fig. 3A and Table S1). Fixed liver sections were stained with H&E and examined at different magnifications (Fig. 3B). It appears that the SKO mice have serious hepatic steatosis. Cryosections of liver were stained with oil red O, and the liver of the SKO mice contained much more lipid droplets than that of WT mice (Fig. 3C). These morphology and histology abnormal were completely recovered in the Seipin-RE mice (Fig. 3A, B and C). We also measured the amount of lipid in the liver. SKO mice have approximately 2-fold triglyceride content than that of WT littermates (Fig. 3D), while Seipin-RE mice were completely normal. There was no difference of cholesterol accumulation in the liver among three groups (Fig. 3D). The mRNA expression of some lipogenic genes such as FAS, PPARγ and SCD1 was significantly increased, while PPARα which was associated with fatty acid oxidation and MTP which was essential for VLDL packaging were decreased in the liver of SKO mice (Fig. 3E). These changes of mRNA expression were restored in Seipin-RE mice. The increased triglyceride accumulation in the liver of SKO mice could be result from insufficient capacity of triglyceride storage in the adipose tissue and overflow to the liver, thus recovered adipose mass in the Seipin-RE mice could restore the lipid deposition in the liver.

3.5. Insulin resistance was normalized in Seipin-RE mice

We evaluated insulin sensitivity and glucose homeostasis in 3-month old mice. Plasma glucose level was increased slightly in SKO mice upon fed and fasted states, Seipin-RE mice was normal (Fig. 4A). Plasma insulin level was elevated dramatically in SKO mice under fed state, and Seipin-RE mice displayed equally level of WT mice (Fig. 4B). Glucose and insulin tolerance tests (GTT, ITT)

after 4-h fasted showed that SKO mice had delayed glucose clearance (Fig. 4C) and impaired insulin sensitivity (Fig. 4D). These indicated that SKO mice suffered severe insulin resistance. In the Seipin-RE mice GTT and ITT were completely normalized (Fig. 4C and D) which illustrated that Seipin-RE mice have restored insulin sensitivity and glucose homeostasis. The mRNA levels of insulin signal related genes such as insulin receptor substrate 1 (IRS1), IRS2, AKT2 and gluconeogenic genes such as PEPCK and G6P1 were measured in the liver (Fig. 4E), and IRS1, IRS2, AKT2, GLUT4 were examined in the WAT (Fig. 4F) on fed state. In SKO mice, the expression of IRS1, IRS2 and AKT2 was significantly reduced and G6P1 was slightly increased in the liver (Fig. 4E), and the expression of all the four genes were markedly decreased in the WAT (Fig. 4F). These results suggest that there are impaired insulin signaling in the liver and WAT of the SKO mice. The change of gene expression in SKO mice was partially restored in Seipin-RE mice.

4. Discussion

Seipin plays an essential role in adipogenesis and lipolysis. We and others have previously demonstrated that loss of seipin in mice results in depletion of adipose tissue, dyslipidemia, with severe hepatic steatosis and insulin resistance [9,15,26]. Recently we reported adipose-specific seipin ablation in mice could lead progressive lipodystrophy and hepatic steatosis and insulin resistance in elder age [11]. In this study, we expressed human seipin gene in the adipose tissue of seipin null mice. As we expected, dyslipidemia, lipodystrophy, hepatic steatosis and insulin resistance were rescued in these mice, suggesting seipin in adipose tissue is responsible for the multiple metabolic phenotypes in SKO mice.

The major functions of adipose tissue are store energy and release NEFA and glycerol according to the energy demand of body. It also functions in the hormonal regulation of energy homeostasis through secreting adipokines such as leptin and adiponectin. Loss of adipose tissue in lipodystrophy is associated with increased prevalence of insulin resistance, dyslipidemia, hypertension, hepatic steatosis and increased predisposition to atherosclerosis [18]. In recent years, many lipodystrophic mouse models (A-ZIP/F mice [19], aP2-nSREBP-1c mice [20], AGPAT2 KO mice [21], aP2-PPARγ KO mice [22] etc) have been generated. All of them suffered completely or partially loss of adipose tissue, insulin resistance and hepatic steatosis. Surgical implantation of adipose tissue could reverse the dyslipidemia, diabetic and hepatic steatosis phenotypes in A-ZIP/F mice [23], but transplantation using leptin-deficient ob/ob adipose tissue had no effect [24]. These findings indicating that normal adipose tissue is essential for the metabolic disorders in A-ZIP/F mice. In this study, Seipin-RE mice have recovered adipose tissue mass, normalized plasma leptin and adiponectin levels. Therefore, the ameliorated metabolic disorders in Seipin-RE mice maybe due to restored adipose tissue mass and function.

Although hypertriglyceridemia is a common feature of BSCL2 patients, SKO mice displayed normal plasma triglyceride and NEFA in fed state, but dramatically decreased upon overnight fasting, while Seipin-RE mice reversed these disorders. Plasma triglyceride levels are regulated by the balance among synthesis, LPL-mediated hydrolysis and hepatic remnants clearance. Plasma triglycerides mainly exist within triglyceride-rich lipoproteins (TRL), i.e. dietary derived chylomicron (CM), liver derived very low-density lipoprotein (VLDL), and their remnant particles [25]. During prolonged fasting, there was no dietary derived CM. We have detected post-heparin LPL activity in WT and SKO mice, and found no significant difference between them. In recently, Prieur et al. observed that hypotriglyceridemia in SKO mice was linked to increased plasma triglyceride clearance through elevated uptake of TRL and NEFA by the liver without impairing VLDL secretion [26].

During fasting, triglycerides in white adipose tissue are hydrolyzed and released as NEFA, then the NEFA was uptake by the liver for oxidation or metabolized to ketone bodies such as β-hydroxybutyrate. SKO mice did not increase their NEFA as WT mice, but paradoxically dropped them upon prolonged fasting. We attributed this to the inability of the SKO mice to replenish their circulating NEFA as a result of the lack of adipose tissue to mobilize. Moreover, the WT mice increased their plasma β-hydroxybutyrate levels upon fasting, in contrast, the SKO mice were unable to do this. While Seipin-RE mice displayed normally NEFA and β-hydroxybutyrate levels and reaction to fasting, which were similar with WT mice. Taken together, these data demonstrated that SKO mice were unable to maintain lipid homeostasis to fasting, due to lack of sufficient mobilizable triglycerides stored in the adipose tissue. Therefore, the normalized lipid profile in Seipin-RE mice maybe result from recovered adipose mass.

AGPAT2 is the causative gene of CGL1. AGPAT2 deficient mice also suffer severe lipodystrophy and hepatic steatosis [21]. Because AGPAT2 is highly expressed in the liver, Agarwal et al. have generated liver specific AGPAT1 or AGPAT2 reconstituted in AGPAT2-KO mice [27]. AGPAT1 or AGPAT2 expression failed to ameliorate the hepatic steatosis in AGPAT2-KO mice, suggesting that the role of AGPAT1 or AGPAT2 in liver lipogenesis was minimal and accumulation of fat in the liver was primarily a consequence of loss of adipose tissue and insulin resistance in AGPAT2-KO mice, excess lipids could no longer be stored in adipose tissues and ectopic accumulated in the liver.

We and others have observed severe hepatic steatosis in SKO mice during fed or short fasted states, while a recent study detected dramatically reduced liver triglyceride content in SKO mice in contrast to increased liver lipid accumulation in WT mice after an overnight fasting [28]. These results suggesting that the liver of SKO mice was able to release its stored triglyceride for other peripheral tissues usage in the energy deficient state. While absence of fat mobilization from adipose tissue resulted in ablation of NEFA flow to the liver, and provided insufficient substrates for fatty acid oxidation, ketone body production and triglyceride accumulation.

We and others also have generated liver-specific seipin knockout mice to study seipin function in the liver [28]. These mice manifested no hepatic steatosis even under high fat diet. In this study, Seipin-RE mice also did not display lipid accumulation in the liver, suggesting that seipin did not play a cellular autonomous role in regulating liver lipid homeostasis in mice. Hepatic steatosis in the lipodystrophic SKO mice was mainly due to insufficient capacity to store triglyceride in the adipose tissue and overflow to the liver.

In conclusion, our study reveals that expression of seipin in adipose tissue rescues lipodystrophy then improves dyslipidemia, insulin resistance and ameliorates hepatic steatosis in SKO mice. These provide clear evidence that seipin expression in adipose tissue is responsible for the multiple metabolic associated disorders in SKO mice.

Conflict of interest

None.

Acknowledgments

This work was supported by the Major National Basic Research Program of the People's Republic of China (No. 2011CB503900) and National Natural Science Foundation of the People's Republic of China (No. 30821001 and 30930037) to George Liu.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.02.147.

References

[1] N. Abate, A. Garg, Heterogeneity in adipose tissue metabolism: causes, implications and management of regional adiposity, Prog. Lipid Res. 34 (1995) 53–70.

[2] A. Garg, Adipose tissue dysfunction in obesity and lipodystrophy, Clin. Cornerstone 8 (Suppl. 4) (2006) S7–S13.

[3] J. Magre, M. Delepine, E. Khallouf, et al., Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13, Nat. Genet. 28 (2001) 365–370.

[4] C. Windpassinger, M. Auer-Grumbach, J. Irobi, et al., Heterozygous missense mutations in BSCL2 are associated with distal hereditary motor neuropathy and silver syndrome, Nat. Genet. 36 (2004) 271–276.

[5] L. Zhou, J. Yin, C. Wang, et al., Lack of seipin in neurons results in anxiety- and depression-like behaviors via down regulation of PPARgamma, Hum. Mol. Genet. 23 (2014) 4094–4102.

[6] M. Jiang, M. Gao, C. Wu, et al., Lack of testicular seipin causes teratozoospermia syndrome in men, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 7054–7059.

[7] V.A. Payne, N. Grimsey, A. Tuthill, et al., The human lipodystrophy gene BSCL2/seipin may be essential for normal adipocyte differentiation, Diabetes 57 (2008) 2055–2060.

[8] W. Chen, V.K. Yechoor, B.H. Chang, et al., The human lipodystrophy gene product BSCL2/Seipin plays a key role in adipocyte differentiation, Endocrinology 150 (2009) 4552–4561.

[9] W. Chen, B. Chang, P. Saha, et al., Berardinelli-seip congenital lipodystrophy 2/seipin is a cell-autonomous regulator of lipolysis essential for adipocyte differentiation, Mol. Cell. Biol. 32 (2012) 1099–1111.

[10] X. Cui, Y. Wang, L. Meng, et al., Overexpression of a short human seipin/BSCL2 isoform in mouse adipose tissue results in mild lipodystrophy, Am. J. Physiol. Endocrinol. Metab. 302 (2012) E705–E713.

[11] L. Liu, Q. Jiang, X. Wang, et al., Adipose-specific knockout of Seipin/Bscl2 results in progressive lipodystrophy, Diabetes 63 (2014) 2320–2331.

[12] W. Fei, G. Shui, B. Gaeta, et al., Fld1p, a functional homologue of human seipin, regulates the size of lipid droplets in yeast, J. Cell. Biol. 180 (2008) 473–482.

[13] K.M. Szymanski, D. Binns, R. Bartz, et al., The lipodystrophy protein seipin is found at endoplasmic reticulum lipid droplet junctions and is important for droplet morphology, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 20890–20895.

[14] J. Bi, W. Wang, Z. Liu, et al., Seipin promotes adipose tissue fat storage through the ER Ca(2+)-ATPase SERCA, Cell. Metab. 19 (2014) 861–871.

[15] X. Cui, Y. Wang, Y. Tang, et al., Seipin ablation in mice results in severe generalized lipodystrophy, Hum. Mol. Genet. 20 (2011) 3022–3030.

[16] L. Fink, W. Seeger, L. Ermert, et al., Real-time quantitative RT-PCR after laser-assisted cell picking, Nat. Med. 4 (1998) 1329–1333.

[17] J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J. Biol. Chem. 226 (1957) 497–509.

[18] I.W. Asterholm, N. Halberg, P.E. Scherer, Mouse models of lipodystrophy key reagents for the understanding of the metabolic syndrome, Drug Discov. Today Dis. Models 4 (2007) 17–24.

[19] J. Moitra, M.M. Mason, M. Olive, et al., Life without white fat: a transgenic mouse, Genes. Dev. 12 (1998) 3168–3181.

[20] I. Shimomura, R.E. Hammer, J.A. Richardson, et al., Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy, Genes. Dev. 12 (1998) 3182–3194.

[21] P. Vogel, R. Read, G. Hansen, et al., Pathology of congenital generalized lipodystrophy in Agpat2-/- mice, Vet. Pathol. 48 (2011) 642–654.

[22] W. He, Y. Barak, A. Hevener, et al., Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 15712–15717.

[23] O. Gavrilova, B. Marcus-Samuels, D. Graham, et al., Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice, J. Clin. Invest 105 (2000) 271–278.

[24] C. Colombo, J.J. Cutson, T. Yamauchi, et al., Transplantation of adipose tissue lacking leptin is unable to reverse the metabolic abnormalities associated with lipoatrophy, Diabetes 51 (2002) 2727–2733.

[25] H.C. Hassing, R.P. Surendran, H.L. Mooij, et al., Pathophysiology of hypertriglyceridemia, Biochim. Biophys. Acta 1821 (2012) 826–832.

[26] X. Prieur, L. Dollet, M. Takahashi, et al., Thiazolidinediones partially reverse the metabolic disturbances observed in Bscl2/seipin-deficient mice, Diabetologia 56 (2013) 1813–1825.

[27] A.K. Agarwal, S. Sukumaran, V.A. Cortes, et al., Human 1-acylglycerol-3-phosphate O-acyltransferase isoforms 1 and 2: biochemical characterization and inability to rescue hepatic steatosis in Agpat2(-/-) gene lipodystrophic mice, J. Biol. Chem. 286 (2011) 37676–37691.

[28] W. Chen, H. Zhou, P. Saha, et al., Molecular mechanisms underlying fasting modulated liver insulin sensitivity and metabolism in male lipodystrophic Bscl2/Seipin-deficient mice, Endocrinology (2014) en20141292.