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Dysfunction of lipid metabolism in lipodystrophic Seipin-deficient mice

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ABSTRACT

Congenital generalized lipodystrophy (CGL) is characterized by a complete loss of body adipose tissue accompanying dyslipidemia, severe hepatic steatosis and insulin resistance. However, the mechanisms of dyslipidemia and hepatic steatosis are unclear. Here using the lipodystrophic Seipin-deficient mouse (*Seipin*$^{-/-}$) model, we found *Seipin*$^{-/-}$ mice were unable to respond appropriately to a long time fasting and developed postprandial hypertriglyceridemia. Impaired very low density lipoprotein (VLDL) secretion and enhanced triglyceride-rich lipoproteins (TRL) clearance were also observed in our *Seipin*$^{-/-}$ mice. To identify the association between upregulation of hepatic LDL receptor and enhanced TRL clearance, we crossed *Seipin*$^{-/-}$ mice with *Ldlr*$^{-/-}$ mice to generate *Seipin*$^{-/-}$*Ldlr*$^{-/-}$ mice. *Seipin*$^{-/-}$*Ldlr*$^{-/-}$ mice displayed increased TRL clearance only after 24 h-fast rather 6 h-fast. In contrast to *Seipin*$^{-/-}$ mice, *Seipin*$^{-/-}$*Ldlr*$^{-/-}$ mice displayed hypertriglyceridemia as observed in human CGL patients. Furthermore, in this study, we demonstrated hepatic steatosis in lipodystrophy *Seipin*$^{-/-}$ mice is a metabolic adaptation of dysfunctional adipose tissue. This study using lipodystrophic model established the importance of adipose tissue in energy homeostasis and lipid metabolism.

1. Introduction

Adipose tissue plays an important role in maintaining energe homeostasis. Obesity and lipodystrophy are characterized by the disproportionate gain or loss of adipose tissue. Both obesity and lipodystrophy are frequently associated with an increase in insulin resistance and its complications [1]. Mouse model of lipodystrophy may provide us with new understanding of adipose tissue.

Congenital generalized lipodystrophy (CGL), characterized by a complete loss of body adipose tissue, is an autosomal recessive disease [2,3]. The most severe form of CGL, type 2 CGL, is caused by mutation in Seipin. Seipin locates in ER and regulates adipocyte differentiation and lipolysis, determining the size and distribution of lipid droplets [4]. As an excellent mouse model for CGL2 lipodystrophy, *Seipin*$^{-/-}$ mice suffered an almost complete loss of white adipose tissue, and a ~60% decrease of brown adipose tissue. These mice also had hepatic steatosis and severe insulin resistance in addition to total absence of white adipose tissue [5,6]. However, in contrast to hypertriglyceridemia in type 2 CGL patients, *Seipin*$^{-/-}$ mice developed hypotriacylglycerolemia upon a long time fasting [5,6,7]. Additionally, imparied postprandial glucose and lipid clearance were also observed in these mice [6]. A recent study reported that the upregulation of hepatic LDL receptor played an important role in increased TRL clearance of *Seipin*$^{-/-}$ mice [7].

In the current study, we took advantage of a novel lipodystrophy model, the *Seipin*$^{-/-}$ mice, to assess the impact of adipose tissue loss on energe homeostasis and lipid metabolism. Impaired VLDL secretion and enhanced TRL clearance were observed in our *Seipin*$^{-/-}$ mice. Generalized *Seipin*$^{-/-}$*Ldlr*$^{-/-}$ mice were used to determine the effect of hepatic LDL receptor in enhanced TRL clearance.

2. Materials and methods

2.1. Animals

Homozygous *Seipin*$^{-/-}$ mice were generated as previously described [5]. *Ldlr*$^{-/-}$ mice were purchased from Jackson Laboratories (Bar Harbor, ME). *Seipin*$^{-/-}$ mice on a C57BL/6J background were crossed with *Ldlr*$^{-/-}$ mice to produce *Seipin*$^{-/-}$*Ldlr*$^{-/-}$ mice. All experiments involving mice were approved by the Institutional

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Animal Care Research Advisory Committee of the National Institute of Biological Science (NIBS) and Animal Care Committee of Peking University Health Science Center. All mice were maintained on a 12:12-h light–dark cycle and fed ad libitum with regular mouse chow diet (10% of kilocalories from fat).

2.2. Blood metabolite analysis

Plasma total cholesterol (TC), triglycerides (TG) and 3-hydroxybutyrate were measured according to manufacturer's protocols (Sigma–Aldrich kit). For lipoprotein distribution analysis, pooled plasma samples from 6 to 8 mice per group were separated by fast protein liquid chromatography (FPLC) and cholesterol and triglycerides were determined in each fraction.

2.3. VLDL secretion

Mice were fasted for 24 h and intravenously injected with 3% Triton 3349 at 800 mg/kg BW. Blood was collected at time 0, 15, 30, and 60 and 120 min after injection. Plasma TG was measured as described above.

2.4. Plasma LPL activity analysis

Mice were fasted for 24 h, blood samples were taken from before and 30 min after heparin injection (1 IU/g.i.p). LPL activity was determined as described [8].

2.5. Fat tolerance and pyruvate tolerance test

For fat tolerance, after mice were fasted for 6 h or 24 h, blood samples were collected after an oral fat load (10 mg/kg BW) by gastric gavage and TG were measured. For pyruvate tolerance test, mice were fasted for 24 h and injected intraperitoneally with pyruvate, plasma glucose level was measured using an enzymatic method (Sigma kits, MO, USA).

2.6. Analysis of lipid content in liver

Approximately a 100 mg piece of liver was weighed and homogenized in 1 mL PBS. Lipids were extracted using Folch's reagent (CHCL3/MeOH, 3:1) [9], and dissolved in 1 ml 3% Triton X-100. Analysis of TG was carried out using enzymatic methods as described above for plasma sample and normalized to tissue weights.

2.7. Morphology of liver tissue

Tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections (2 μm) were stained with hematoxylin and eosin (H&E).

2.8. Western blot analysis, RNA isolation and quantitative real-time PCR

Western blot analysis, RNA isolation and quantitative real-time PCR were performed as described [10].

2.9. Statistical analysis

All data are presented as means ± SEM. Statistical comparison between groups was performed using Student's t-test. A value of $P < 0.05$ was considered statistically significant.

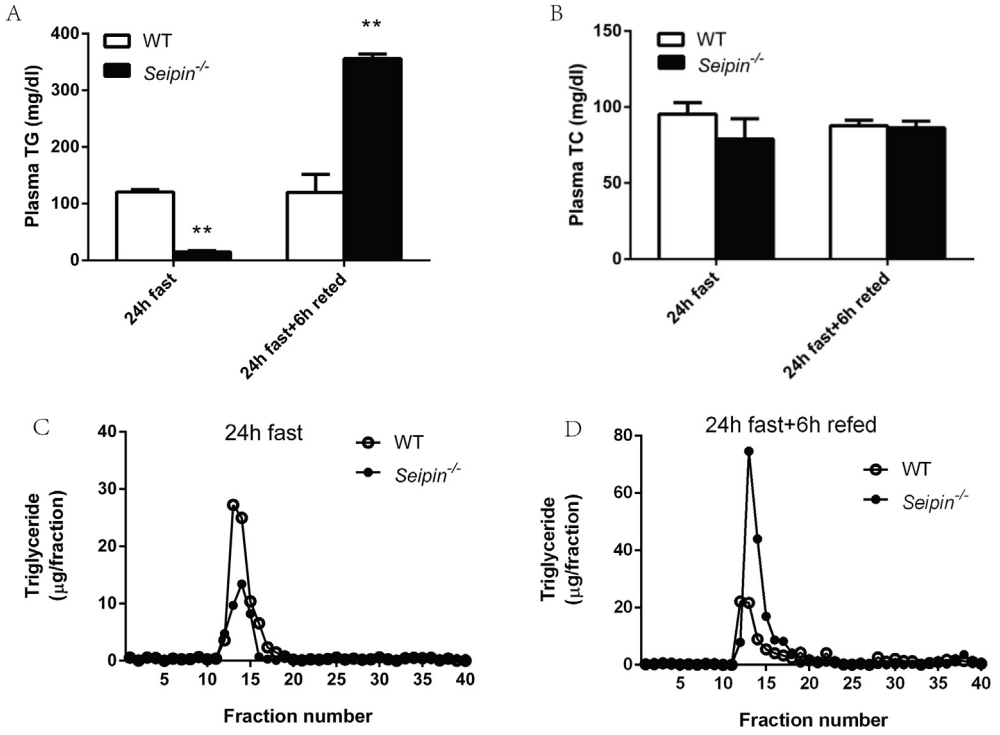

Fig. 1. Postprandial hypertriglyceridemia in Seipin$^{-/-}$ mice. A. Plasma TG level in 24 h fast mice and 24 h fast followed by 6 h refed mice; B. Plasma TC level in 24 h fast mice and 24 h fast followed by 6 h refed mice; C,D. Plasma lipoprotein distribution analysis of triglycerides in 24 h fasted (C) and 24 h fast followed by 6 h refed (D)*Seipin*$^{-/-}$ and WT mice (n = 6). Values are expressed as mean ± SEM. **P < 0.005 for *Seipin*$^{-/-}$ vs. WT mice.

3. Results

3.1. Postprandial hypertriglyceridemia in *Seipin*$^{-/-}$ mice

We and others noticed a dramatic decrease in plasma TG level in 24 h-fasted *Seipin*$^{-/-}$ mice [4,6,7]. But when the 24 h-fasted *Seipin*$^{-/-}$ mice were refed normal chow diet for 6 h, plasma TG reached much higher level in *Seipin*$^{-/-}$ mice than in WT mice (Fig. 1A). No obvious changes were observed in plasma TC level (Fig. 1B). Gel filtration chromatography revealed that TG contained in the very low density lipoprotein (VLDL)/chylomicron increased significantly in *Seipin*$^{-/-}$ mice (Fig. 1C), suggesting that Seipin deficiency might result in triglyceride-rich chylomicrons and VLDLs accumulation in the blood.

3.2. Impaired VLDL secretion and enhanced TRL clearance in *Seipin*$^{-/-}$ mice

We studied VLDL-TG secretion rate using the lipoprotein lipase inhibitor triton1339. 24 h-fast *Seipin*$^{-/-}$ mice had a decreased VLDL secretion rate as compared to WT mice (Fig. 2A). Accordingly, plasma apoB48 content significantly decreased at each time point after intravenous injection of triton 1339 (Fig. 2A). We also studied the ability of mice to clear fat administered via gastric gavage. 24 h-fast *Seipin*$^{-/-}$ mice displayed enhanced TRL clearance (Fig. 2B). Furthermore, this phenotype had no relationship with plasma lipase activity (Fig. 2C). Recently, Prieur et al. reported that the upregulation of hepatic LDL receptor in *Seipin*$^{-/-}$ mice is a key contributor to increased TRL clearance [7]. We also observed an increased level of hepatic LDL receptor (Fig. 2D), which we hypothesized might be a metabolic adaptation to starvation in *Seipin*$^{-/-}$ mice. To test this, we generated the *Seipin*$^{-/-}$*Ldlr*$^{-/-}$ mice. After fasted 24 h, *Seipin*$^{-/-}$*Ldlr*$^{-/-}$ mice displayed increased TRL clearance as observed in *Seipin*$^{-/-}$ mice (Fig. 2E). However when fasted for 6 h, the plasma TG of *Seipin*$^{-/-}$*Ldlr*$^{-/-}$ mice remained a relatively higher level than *Ldlr*$^{-/-}$ mice (Fig. 2F), indicating a compromised TG clearance ability. Hypertriglyceridemia is a common feature of CGL patients [11], however, *Seipin*$^{-/-}$ mice displayed relatively decreased plasma TG level [5,6]. Here, *Seipin*$^{-/-}$*Ldlr*$^{-/-}$ mice exhibited increased plasma TG and TC (~400 mg/dl and

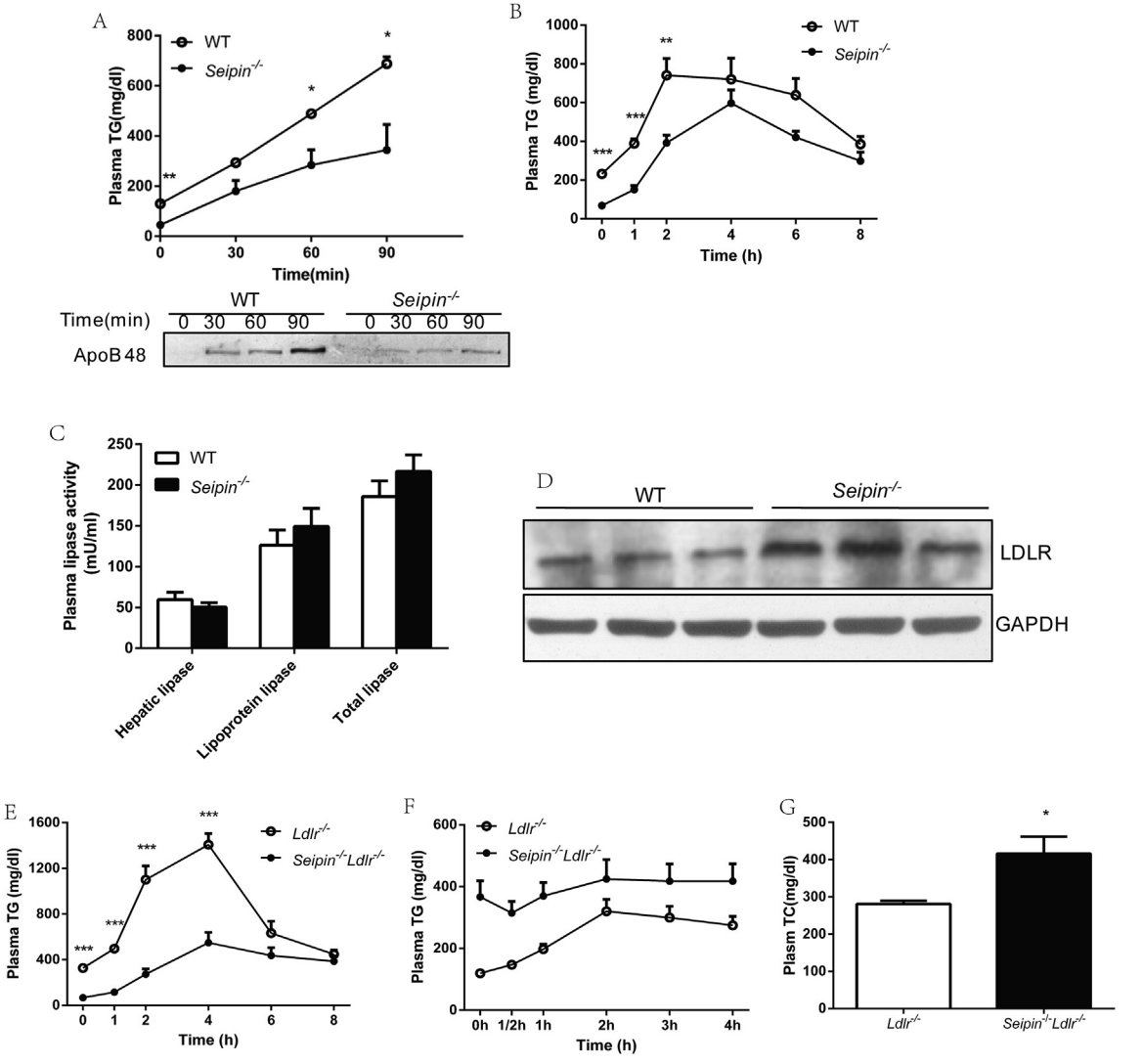

Fig. 2. Impaired VLDL secretion and enhanced TRL clearance in *Seipin*$^{-/-}$ mice A. VLDL-TG secretion in *Seipin*$^{-/-}$ mice. Blood sample were collected at the indicated durations after Triton WR-1339 injection. Levels of plasma apoB-48 were detected by immunoblotting. B. TRL clearance in 24 h-fast *Seipin*$^{-/-}$ and WT mice. C. Plasma lipase acitivity in *Seipin*$^{-/-}$ and WT mice. D. Representative Western Blot images for hepatic LDLR protein. E, F. TRL clearance in 24 h-fast *Seipin*$^{-/-}$ *Ldlr*$^{-/-}$ (E) and 6 h-fast *Seipin*$^{-/-}$ *Ldlr*$^{-/-}$ mice (F). G. Plasma TC level in 6 h-fast *Seipin*$^{-/-}$ *Ldlr*$^{-/-}$ and *Ldlr*$^{-/-}$ mice. Values are expressed as mean ± SEM. *P < 0.05, **P < 0.005 for *Seipin*$^{-/-}$ vs. WT mice or *Seipin*$^{-/-}$ *Ldlr*$^{-/-}$ vs. *Ldlr*$^{-/-}$ mice.

~450 mg/dl, respectively) (Fig. 2F and G) as compared to their control mice.

3.3. Fasting induces metabolic adaptation in liver of *Seipin*$^{-/-}$ mice

We further analyzed hepatic lipid profiles of *Seipin*$^{-/-}$ and WT mice under fed and 24-h fast condition. 24-h fast induced numerous lipid droplets (Fig. 3A) and a nearly 2-fold increase in liver TG accumulation in 24-h fasted WT mice (Fig. 3B). Severe hepatic steatosis in fed *Seipin*$^{-/-}$ mice was observed, whereas 24-h fast induced a decrease of lipid droplets in *Seipin*$^{-/-}$ mice (Fig. 3A). To figure out the mechanism of decreased lipid accumulation induced by long time fasting, we detected gene expression of hepatic adipose triglyceride lipase (ATGL), which is the major

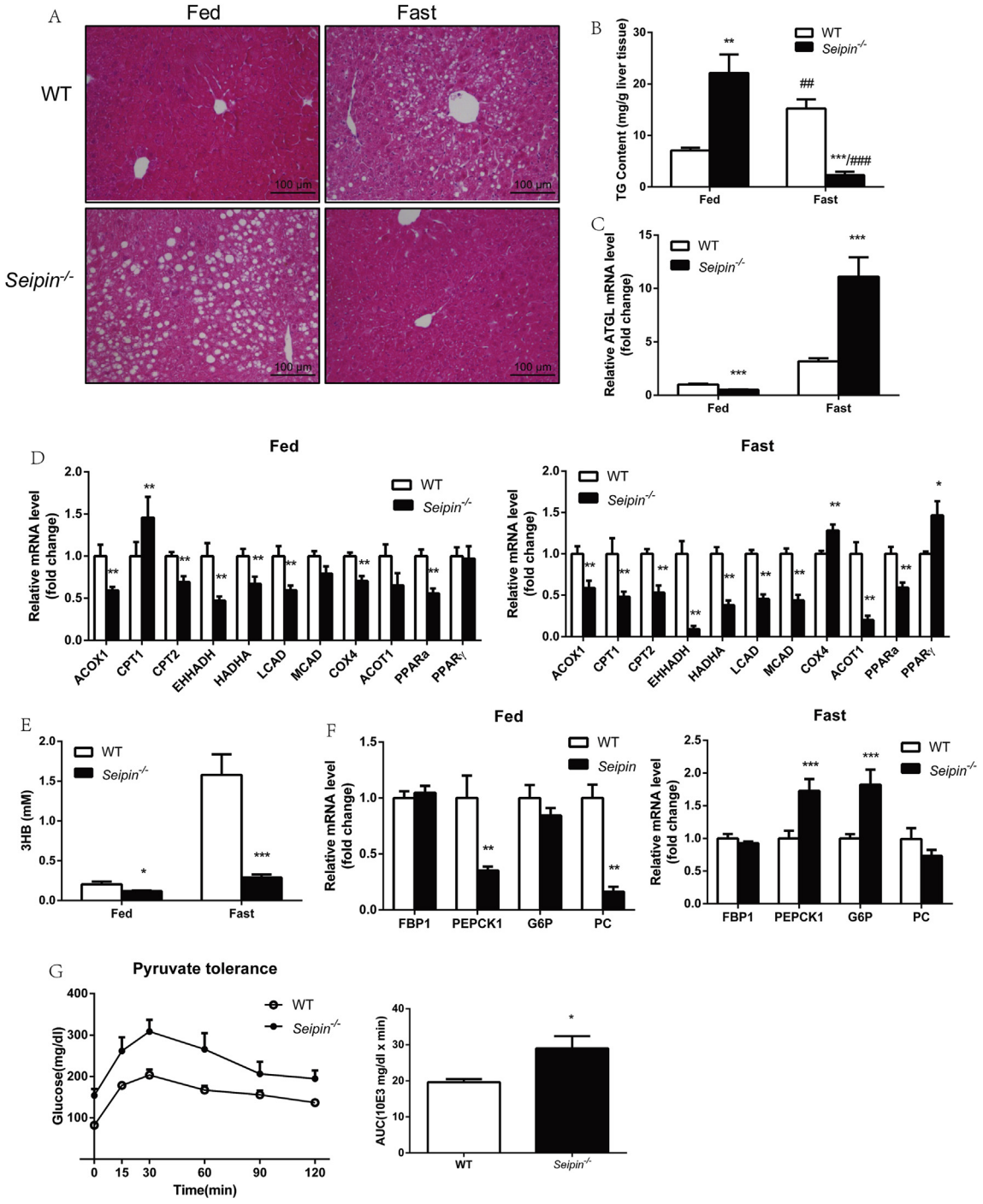

Fig. 3. Fasting induces metabolic adaptation in liver of *Seipin*$^{-/-}$ mice A. HE staining of liver from WT and *Seipin*$^{-/-}$ mice in fed and fast state; B. TG contents of the liver from WT and *Seipin*$^{-/-}$ mice in fed and fast state; C. ATGL gene expression in the liver from WT and *Seipin*$^{-/-}$ mice in fed and fast state. Values are fold induction of gene expression normalized to the housekeeping gene GAPDH. D,E. β-oxidation related genes expression (D) and plasma β-hydroxybutyrate (3-HB) levels of WT and *Seipin*$^{-/-}$ mice in fed and fast state. F. Gluconeogenesis related genes expression in the liver of WT and *Seipin*$^{-/-}$ mice. G. Pyruvate tolerance test, after a 24 h fast, mice were injected with 2 g/kg pyruvate and blood glucose levels were detected at indicated times (n = 6 per group). Area under curve (AUC) is also quantified (right). Values are expressed as mean ± SEM. N = 6–8, $^{*}P < 0.5$,$^{**}P < 0.005$,$^{***}P < 0.001$ for *Seipin*$^{-/-}$ vs. WT mice.

cytoplasmic TG hydrolase in the liver. ~3fold increase of ATGL gene expression was detected in *Seipin*$^{-/-}$ mice (Fig. 3C), which might be an important contributor to reduction of TG accumulation. To determine whether enhanced FA oxidation contributed to the reduction in TG accumulation, we measured FA oxidation gene expression and the plasma levels of ketone body 3-hydroxybutrate. As revealed by real time PCR analysis, gene related to FA oxidation (ACOX1, CPT2, EHHADH, HADHA, LCAD, MCAD, ACOT1, PPARα) decreased significantly in both fed and 24-h fast condition (Fig. 3D). Our result suggested a markedly reduced plasma concentration of 3-hydroxybutrate in *Seipin*$^{-/-}$ mice compared to WT mice after a 24-h fast (Fig. 3E). The mRNA expression of gluconeogenic enzymes (PEPCK1 and PC) was reduced in fed condition (Fig. 3F). After a 24-h fast, PEPCK1, PC and G6P mRNA were upregulated. Pyruvate tolerance was subjected after a 24-h fast. The fasted *Seipin*$^{-/-}$ mice displayed a greater increase in plasma glucose levels than WT mice at each time points (Fig. 3G). A recent study revealed no detectable Seipin protein in mouse hepatocytes [12], and liver specific Seipin knockout mice did not show any changes in plasma lipid (data not shown). Our data demonstrated that, due to the lack of sufficient mobilizable lipid stores, *Seipin*$^{-/-}$ mice were unable to respond appropriately to a long time fast.

4. Discussion

Lipodystrophy, the other end of the obesity, is also associated with many metabolic disturbances in human patients, for example, dyslipidemia, hepatic steatosis and insulin resistance. These combined metabolic disorders put an individual at increased risk of cardiovascular diseases and diabetes. Recent studies using lipodystrophic mouse model have established the importance of adipose tissue in these metabolic disorders [5,7,13]. However the relationship between lipodysrophy and dyslipidemia and hepatic steatosis remains to be elucidated. In the present study, we demonstrated that dyslipidemia and hepatic steatosis of lipodystrophic model were a metabolic reflection of adipose tissue absence.

Seipin$^{-/-}$ mice are an excellent model for lipodystrophy. Although hypertriglyceridemia is a common lipid metabolic disorder in patients with lipodystrophy [14,15,16], previous studies have demonstrated *Seipin*$^{-/-}$ mice exhibits hypotriacylglycerolemia after a prolonged fasting, these findings suggest a possible metabolic difference between mice and humans. Generalized *Seipin*$^{-/-}$*Ldlr*$^{-/-}$ mice exhibited hypertriglyceridemia only in a short time fasting state. Our current animals might provide an excellent model more relevant to human lipodystrophy.

In contrast to obviously increased hepatic lipid accumulation induced by a long time fast in WT mice, we and another group [13] observed a significantly reduced hepatic TG accumulation in *Seipin*$^{-/-}$ mice. Due to lack of sufficient mobilizable energy from adipose tissue, liver can adaptively release its stored TG hydrolyzed by ATGL. Considering the notion that there is no detectable Seipin expression in liver, these results indicated the increased lipid disposition in liver of *Seipin*$^{-/-}$ mice might be an adaptation reflection of adipose tissue dysfunction. Enhanced TRL clearance in 24 h-fast *Seipin*$^{-/-}$ mice might be a metabolic adaptation to energy shortage, despite upregulation of hepatic LDL receptor was observed in our *Seipin*$^{-/-}$ mice. To identify the contribution of hepatic LDL receptor upregulation in enhanced TRL clearance, we detected TRL clearance of *Seipin*$^{-/-}$*Ldlr*$^{-/-}$ mice both in short time fasting and long time fasting. Our results showed only in a long time fasting, *Seipin*$^{-/-}$*Ldlr*$^{-/-}$ displayed increased TRL clearance. Most recently, two other groups reported similar [7] or increased [13] VLDL secretion, however, we found impaired VLDL secretion consistent with decreased apoB48 expression in our *Seipin*$^{-/-}$ mice. We speculated the discrepancy may be related to different fast timing and different LPL activity blocker.

In conclusion, absence of adipose tissue in our lipodystrophic *Seipin*$^{-/-}$ mouse model resulted in inappropriate response to long time fasting (impaired VLDL secretion, enhanced TRL clearance) and a large amount of TG accumulation in liver upon fed or short time fast state. Our findings highlighted adipose tissue as an essential organ for proper metabolic regulation. In particular, hepatic steatosis in lipodystrophy caused by ectopic lipid disposition is a metabolic adaptation reflection of dysfunctional adipose tissue.

Conflict of interest

All authors declare that there are no competing financial interests in relation to the work described.

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