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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.

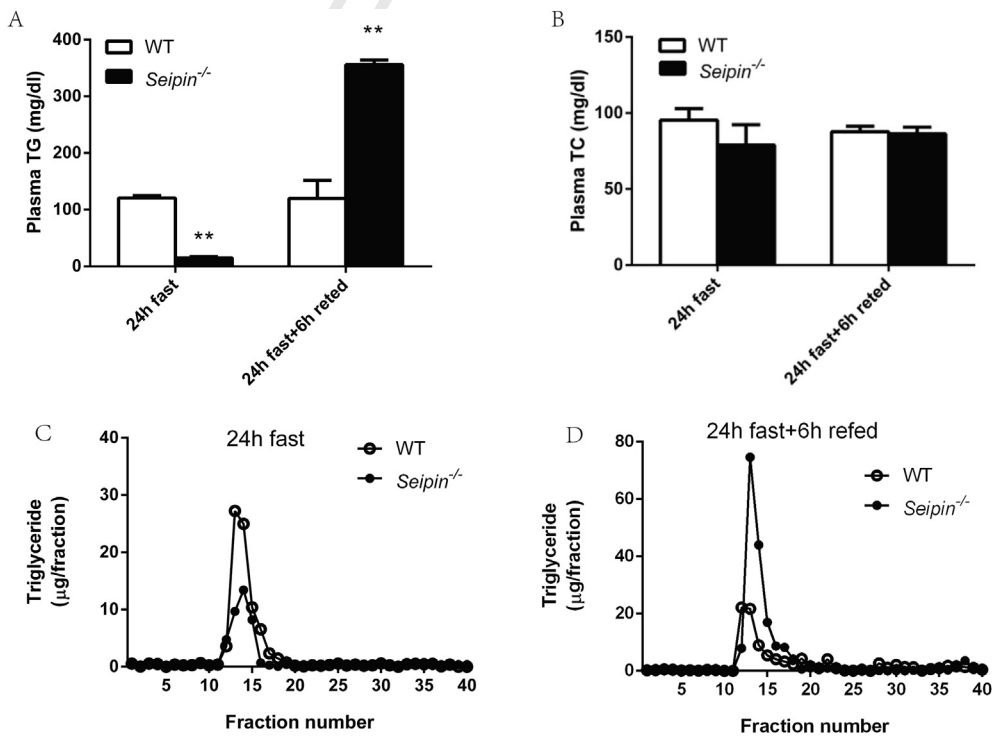

Fig. 1. Postprandial hypertriglyceridemia in Seipin$^{-/-}$ mice. A. Plasma TG level in 24 h fast mice and 24 h fast followed by 6 h refed mice; B. Plasma TC level in 24 h fast mice and 24 h fast followed by 6 h refed mice; C,D. Plasma lipoprotein distribution analysis of triglycerides in 24 h fasted (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.

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