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Ablation of PPP1R3G reduces glycogen deposition and mitigates high-fat diet induced obesity

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ABSTRACT

Glycogen and triglyceride are two major forms of energy storage in the body and provide the fuel during different phases of food deprivation. However, how glycogen metabolism is linked to fat deposition in adipose tissue has not been clearly characterized. We generated a mouse model with whole-body deletion of PPP1R3G, a glycogen-targeting subunit of protein phosphatase-1 required for glycogen synthesis. Upon feeding with high-fat diet, the body weight and fat composition are significantly reduced in the PPP1R3G$^{-/-}$ mice compared to the wild type controls. The metabolic rate of the mice as measured by O_2 consumption and CO_2 production is accelerated by PPP1R3G deletion. The high-fat diet-induced liver steatosis is also slightly relieved by PPP1R3G deletion. The glycogen level in adipose tissue is reduced by PPP1R3G deletion. In 3T3L1 cells, overexpression of PPP1R3G leads to increases of both glycogen and triglyceride levels. In conclusion, our study indicates that glycogen is actively involved in fat accumulation in adipose tissue and obesity development upon high-fat diet. Our study also suggests that PPP1R3G is an important player that links glycogen metabolism to lipid metabolism *in vivo*.

1. Introduction

Glycogen and triglyceride are two major forms of energy storage in the body and provide fuel needed during different phases of food deprivation. After a meal, glucose is converted into glycogen primarily in the skeletal muscle and liver to maintain glucose homeostasis, while the glycogen stores represent a major energy source to protect against hypoglycemia during the early phase of food deprivation (Cherrington, 1999). The synthesis and degradation of glycogen are both regulated by hormones and metabolic signals mainly via modulating the enzymatic activities of glycogen synthase (GS) and glycogen phosphorylase (GP) (Agius, 2008). GS catalyzes the addition of glucose to the glycogen chain, and GP catalyzes the breakdown of glycogen to release glucose-1-phosphate. Phosphorylation of GS leads to inhibition of the enzyme activity, with its multiple residues being phosphorylated by a variety of protein kinases (Ceulemans and Bollen, 2004; Brady and Saltiel, 2001). On the other hand, the activity of GS is stimulated by dephosphorylation via glycogen synthase phosphatase (GSP). In contrast to GS, the activity of GP is activated by phosphorylation on one N-terminal serine by phosphorylase kinase and inhibited by dephosphorylation by protein phosphatase 1 (PP1) (Ceulemans and Bollen, 2004; Brady and Saltiel, 2001). After a meal, the elevated glucose promotes the dephosphorylation of GP and inhibits its activity, and GSP is then released from the allosteric inhibitory effect of glycogen phosphorylase a (GPa) (Nuttall et al., 1988). In addition, glycose-6-phosphate (G6P) is an allosteric activator of GS, serving to make GS a better substrate for dephosphorylation and activation by protein phosphatases (Villarpalasi, 1991; Shulman and Rothman, 1996). After a meal, the increase in intracellular G6P concentration results in an increase in GS activity and inactivation of GP. In addition, the elevated insulin after a meal contributes to stimulation of GS activity by inhibition of glycogen synthase kinase-3 (GSK3) (Hughes et al., 1993). Through these complex processes, feeding leads to activation of GS and inactivation of GP, resulting in glycogen accumulation.

Glycogen-targeting regulatory subunits (G subunits) coordinate glycogen synthesis by targeting the catalytic subunit of PP1 to the glycogen particles (Agius, 2008; Ceulemans and Bollen, 2004). They modulate the activities of the glycogen-metabolizing enzymes

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through PP1-mediated dephosphorylation, functioning as a major GSP to dephosphorylate and activate GS and in turn, stimulate glycogenesis. There are seven genes encoding G subunits (PPP1R3A to PPP1R3G), according to the GenBank database (Ceulemans and Bollen, 2004). PPP1R3A (GM) is mainly expressed in the skeletal muscle and heart with deletion of this gene leading to reduction of glycogen level in skeletal muscle, accompanied by glucose intolerance and insulin resistance (Delibegovic et al., 2003). PPP1R3B (GL) is the primary G subunit expressed in the liver and overexpression of a deregulated form of PPP1R3B in mice displayed improved glucose tolerance (Kelsall et al., 2009). PPP1R3C (PPP1R5) is expressed in many tissues and mice with heterozygous deletion of PPP1R3C in mice led to reduction of glycogen levels in many tissues, accompanied by progressive glucose intolerance, hyperinsulinemia and insulin resistance with aging (Crosson et al., 2003). PPP1R3D (PPP1R6) is mainly expressed in the brain and likely plays a function in glycogen accumulation in neurons (Rubio-Villena et al., 2013). PPP1R3E is highly expressed in the liver and heart muscle in rodents (Munro et al., 2005). PPP1R3F is a membrane-associated G subunit and has been reported to regulate glycogen synthase in astrocytoma cells (Kelsall et al., 2011). PPP1R3G is the seventh glycogen-targeting regulatory subunit of PP1 and it is expressed at the fast-to-fed transition in the mouse liver and plays an important role in postprandial glucose homeostasis (Luo et al., 2011). Collectively, these data indicate that the G subunits play important physiological functions in glycogen and glucose metabolisms in various tissues.

To further investigate the physiological function of PPP1R3G, we generated a mouse model with whole-body deletion of PPP1R3G. Strikingly, we found that PPP1R1G has a unique role in modulating obesity *in vivo*, thus indicating a novel physiological role of glycogen in lipid metabolism.

2. Results

2.1. Characterization of PPP1R3G gene knockout mice

Our previous studies revealed that PPP1R3G, as a glycogen-targeting regulatory subunit of protein phosphatase 1 (PP1), is able to regulate glucose metabolism (Luo et al., 2011; Zhang et al., 2014). In this study, we further explored the *in vivo* function of PPP1R3G with a mouse model that had a whole-body deletion of PPP1R3G gene. The whole coding region of PPP1R3G gene is localized in exon 2 which was replaced by a neomycin cassette (Fig. 1A). As PPP1R3G was expressed in many mouse tissues with a relatively high level in the liver, brain and white adipose tissue (Luo et al., 2011), we analyzed the expression of PPP1R3G in the mouse brain. As shown in Fig. 1B, homozygous knockout of PPP1R3G gene led to a complete deletion of PPP1R3G protein in the brain. We also analyzed the expression of PPP1R3G in the liver. PPP1R3G is a cyclic gene that changes along with the fasting–feeding cycle in the mouse liver (Luo et al., 2011). As expected, PPP1R3G protein was induced by fasting in the wild-type mice liver, while no PPP1R3G protein was detected in the liver of PPP1R3G$^{-/-}$ mice upon fasting (Fig. 1C). Collectively, these data confirmed that PPP1R3G gene was successfully deleted in our mouse model.

2.2. PPP1R3G deletion reduces high fat diet-induced obesity

Under the condition of normal chow, the body weight and food intake of PPP1R3G$^{-/-}$ mice were not different from that of the wild type mice (Y.C., data not shown). We next investigated the effect of PPP1R3G deletion on obesity induced by high-fat diet. Six-week-old wild-type and PPP1R3G$^{-/-}$ mice were fed with a high-fat diet for 20 weeks. Compared to the control mice, the body weight of the PPP1R3G$^{-/-}$ mice gained less weight at most time points except for the 1st, 8th,9th, 10th, and 12th week (Fig. 2A). Such reduction of obesity in the PPP1R3G$^{-/-}$ mice was apparently not caused by a decrease in food intake, as food consumption in PPP1R3G$^{-/-}$ mice was even higher than that of wild-type mice (Fig. 2B). The weight of the epididymal fat pad was also significantly reduced in the PPP1R3G$^{-/-}$ mice in comparison with the wild-type mice (Fig. 2C). The percentage of fat pad weight vs body weight was 4.71% in wild type mice and 3.31% in the PPP1R3G$^{-/-}$ mice. We next analyzed the body composition by nuclear magnetic resonance. The fat mass was significantly lower in the PPP1R3G$^{-/-}$ mice (16.7%) than the wild-type mice (27.1%) (Fig. 2D). Consistently, the lean mass of the PPP1R3G$^{-/-}$ mice was higher than that of the wild-type mice (Fig. 2E).

We also analyzed the metabolic rate of the mice using a metabolic cage. Consistent with the reduction of obesity in the PPP1R3G$^{-/-}$ mice, the metabolic rate shown as O_2 consumption and CO_2 production was significantly elevated in these mice (Fig. 3). However, RER was not altered by PPP1R3G deletion (Fig. 3), indicating that the energy source consumed by the mice was not altered by PPP1R3G deletion. Interestingly, the activity of the mice was also not changed by PPP1R3G deletion (Fig. 3). Collectively, these data suggested that PPP1R3G deletion renders the mice resistant to high fat diet-induced obesity together with altered metabolic rate.

2.3. PPP1R3G deletion accelerates postprandial blood glucose clearance

We also analyzed the effect of PPP1R3G deletion on glucose clearance and insulin sensitivity under the condition of high-fat diet. There was no difference in the glucose tolerance test (GTT) between the two group of animals (Fig. 4A), indicating that glucose tolerance was not altered by PPP1R3G deletion. Insulin tolerance test (ITT) revealed that the blood glucose levels in PPP1R3G$^{-/-}$ mice were reduced before and after insulin administration as compared to the wild-type animals (Fig. 4B). We also performed a food tolerance test (FTT) to investigate the effect of PPP1R3G on postprandial blood glucose clearance. The mice were fasted overnight and then refed for different lengths of time. Intriguingly, we observed that the PPP1R3G$^{-/-}$ mice had an increase in postprandial glucose clearance in comparison with the wild-type animals, as the blood glucose levels after feeding were markedly reduced by PPP1R3G deletion (Fig. 4C). Collectively, these data indicated that under high-fat diet, PPP1R3G deletion is associated with acceleration of postprandial blood glucose clearance.

2.4. PPP1R3G deletion slightly decreases high-fat diet-induced hepatic steatosis

High-fat diet could not only induce obesity, but also leads to development of hepatic steatosis. We carefully analyzed the effect of PPP1R3G deletion on fatty liver formation under high fat diet. The glycogen level in the liver was significantly reduced by PPP1R3G deletion (Fig. 5A), shown as 19.1 mg/g in the wild-type mice and 10.9 mg/g in the PPP1R3G$^{-/-}$ animals. The weight of the liver was not significantly different between the two groups of animals (Fig. 5B). However, the hepatic triglyceride level in the PPP1R3G$^{-/-}$ mice was slightly lower than that in control mice (Fig. 5C, $P = 0.078$). Oil Red O staining demonstrated that lipid accumulation in the liver was markedly reduced by PPP1R3G deletion (Fig. 5D). These data, therefore, indicated that PPP1R3G deletion is able to reduce high fat diet-induced fatty liver formation to a certain degree.