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

Yongxian Zhang [1], Jin Gu [1], Lin Wang, Zilong Zhao, Yi Pan, Yan Chen*

CAS Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, China

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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

* Corresponding author. Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yueyang Rd., New Life Science Building, A2214, Shanghai, 200031, China.
E-mail address: ychen3@sibs.ac.cn (Y. Chen).
[1] Both authors contribute equally to this work.

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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.

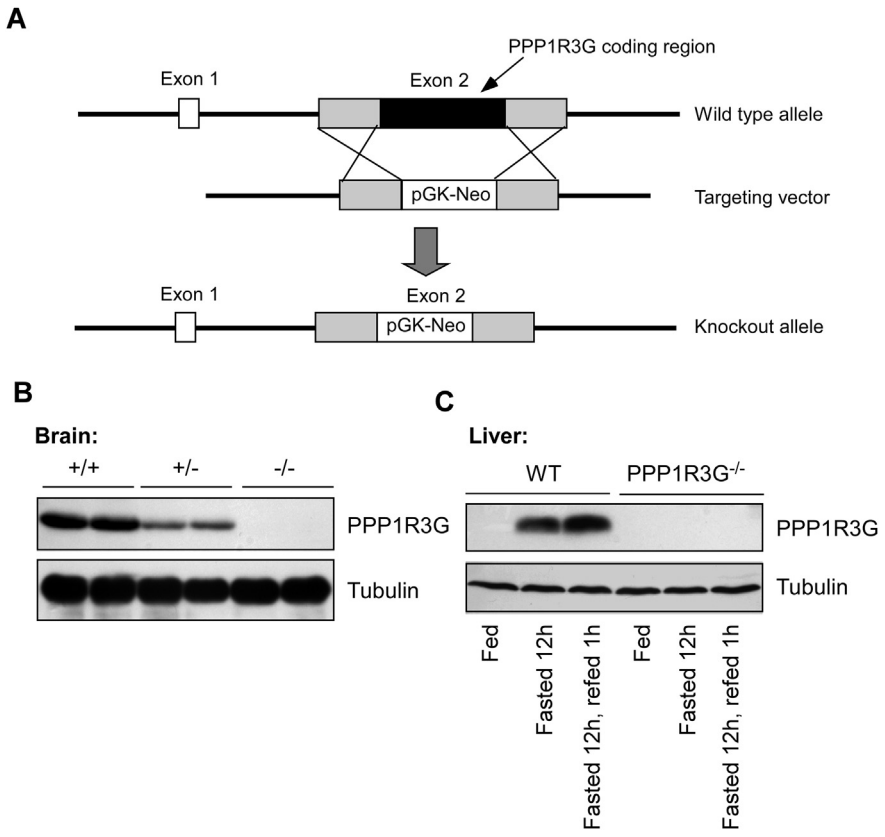

Fig. 1. Generation and characterization of mice with whole body knockout of PPP1R3G gene. (A) A diagram depicting the PPP1R3G knockout construct. The whole mRNA coding region of PPP1R3G gene is localized in exon 2 that is replaced by a neomycin cassette, leading to complete loss of PPP1R3G function after homozygous recombination. (B) PPP1R3G protein is lost in the brain of PPP1R3G$^{-/-}$ mice. The whole brains from the wild-type (WT) as well as heterozygous and homozygous knockout (KO) mice were used in immunoblotting with an antibody against PPP1R3G. (C) Fasting-induced expression of PPP1R3G in the liver is lost in the knockout mice. The livers isolated from the mice under various fasting and feeding conditions were used in immunoblotting to detect PPP1R3G protein.

2.5. Glycogen affects triglyceride accumulation in adipocytes

We next tried to explore the mechanism underlying the reduction of obesity by PPP1R3G deletion under high-fat diet. We hypothesized that the PPP1R3G-mediated glycogen accumulation in the adipose tissue is required for fat storage, as suggested by a few previous studies (Tuerkischer and Wertheimer, 1942; Birsoy et al., 2008). Consistent with the observation that PPP1R3G protein was highly expressed in the white adipose tissue (Fig. 6A), PPP1R3G deletion led to significant reduction of glycogen concentration in epididymal fat pad (Fig. 6B).

We next used 3T3L1 cells to further investigate the role of PPP1R3G in lipid metabolism of the adipocytes. The level of glycogen was dramatically elevated during the differentiation of 3T3L1 cells (Fig. 6C). These cells were then infected with adenovirus containing either the control or PPP1R3G-overexpression plasmid. We found that overexpression of PPP1R3G could significantly elevate the glycogen level after the differentiation of 3T3L1 cells (Fig. 6D). Consistent with our hypothesis, the triglyceride level was significantly enhanced by overexpression of PPP1R3G after 3T3L1 cell differentiation (Fig. 6E). These data indicated that the increased glycogen level in adipocytes could lead to an increase in triglyceride synthesis, explaining our observation that the reduced glycogen accumulation in adipose tissue is associated with reduced obesity in the PPP1R3G$^{-/-}$ mice.

3. Discussion

In this study, we investigated the *in vivo* function of PPP1R3G using a whole-body knockout mouse model in which PPP1R3G was deleted in all tissues. We found that PPP1R3G$^{-/-}$ mice had reduced body weight, decreased fat composition, increased metabolic rate, and mitigated liver steatosis under high-fat diet, suggesting a novel physiological function of PPP1R3G in linking glycogen metabolism to lipid metabolism *in vivo* under the condition of high-fat diet.

How glycogen metabolism is bridged to fat storage in adipose tissue has not been comprehensively investigated. Tuerkischer and Wertheimer in 1942 discovered that a noticeable amount of glycogen is formed in the adipose tissue during the transition of fasted to the fed state (Tuerkischer and Wertheimer, 1942). They speculated that "(1) that adipose tissue can synthesize glycogen, (2) that the glycogen metabolism of adipose tissue is specifically regulated, (3) that adipose tissue can probably effect the conversion of carbohydrate into fat, and (4) that adipose tissue thus appears to play a more active part in carbohydrate-fat metabolism than has hitherto been assumed" (Tuerkischer and Wertheimer, 1942). They also postulated that glycogen deposition is a prerequisite for the conversion of carbohydrate into fat in adipose tissues, as the deposition of fat occurred after the initial glycogen deposition in dynamics (Tuerkischer and Wertheimer, 1942). It was later found that adipose tissue does have a great capacity to synthesize glycogen. In addition to PPP1R3G, PTG (PPP1R3B) is another glycogen-targeting subunit of PP1 expressed in adipocyte tissue

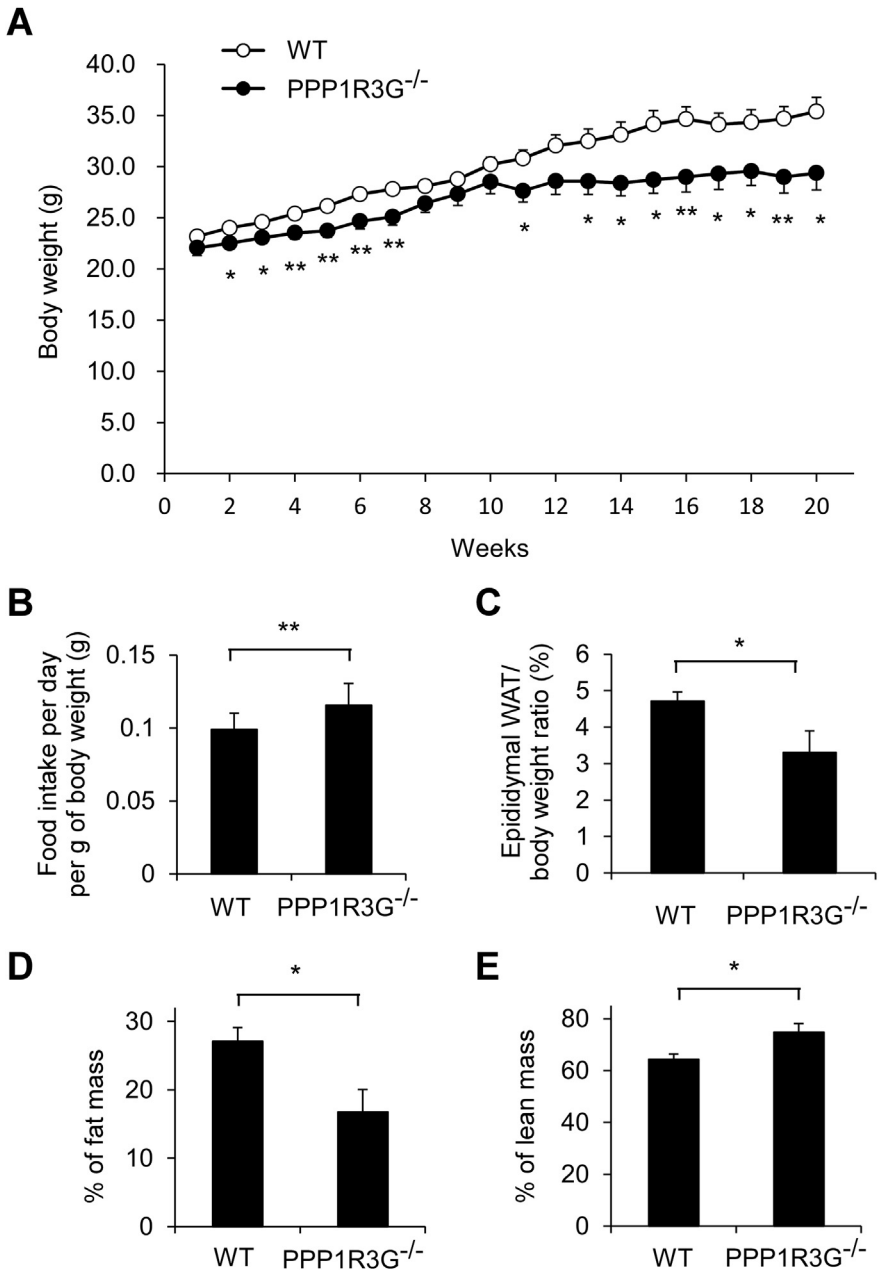

Fig. 2. Deletion of PPP1R3G reduces high-fat diet-induced obesity. Six-week-old male wild type and PPP1R3G$^{-/-}$ mice were fed with HFD for 20 weeks (n = 9 for each group). The body weight (A), food intake (B), epididymal fat weight (C), as well as the percentage of fat mass (D) and lean mass (E) are analyzed with the mice. The data are shown as mean ± SD. * for $P < 0.05$ and ** for $P < 0.01$.

(Printen et al., 1997). Overexpression of PTG in the adipose tissue using an aP2 promoter in a transgenic mouse model demonstrated that the adipose glycogen level is 200- to 400- fold higher in the transgenic mice than that of wild-type mice, revealing that adipocytes are capable of spatially accommodating for a very high glycogen level (Jurczak et al., 2007).

The importance of glycogen for fat deposition in adipose tissue was recently documented by the work of Birsoy et al. (2008). This study demonstrated that the adipocyte tissue is able to shift energy flux dramatically favoring the synthesis of glycogen prior to the re-establishment of lipogenesis. It provided another evidence that glycogen may serve as a pool to provide the substrate for the expansion of adipose tissue from carbohydrate. The Birsoy study led to the speculation that "glycogen metabolism may function as a metabolic switch, helping to re-set the lipogenic program in the adipocyte during the fasted to fed transition" as recently summarized by Markan et al. (Markan et al., 2010).

Our study indicates that PPP1R3G is involved in glycogen synthesis in the adipose tissue and such function contributes to fat deposition and obesity development under the condition of high-fat diet. PPP1R3G is highly expressed in the adipose tissue (Fig. 6A). The glycogen level in the adipose tissue is reduced by about 30% in the PPP1R3G$^{-/-}$ mice under high-fat diet (Fig. 6B). In the same time, the body weight and fat composition are significantly reduced by PPP1R3G deletion under high-fat diet (Fig. 2). We believe that the observed increase in metabolic rate (Fig. 3) and

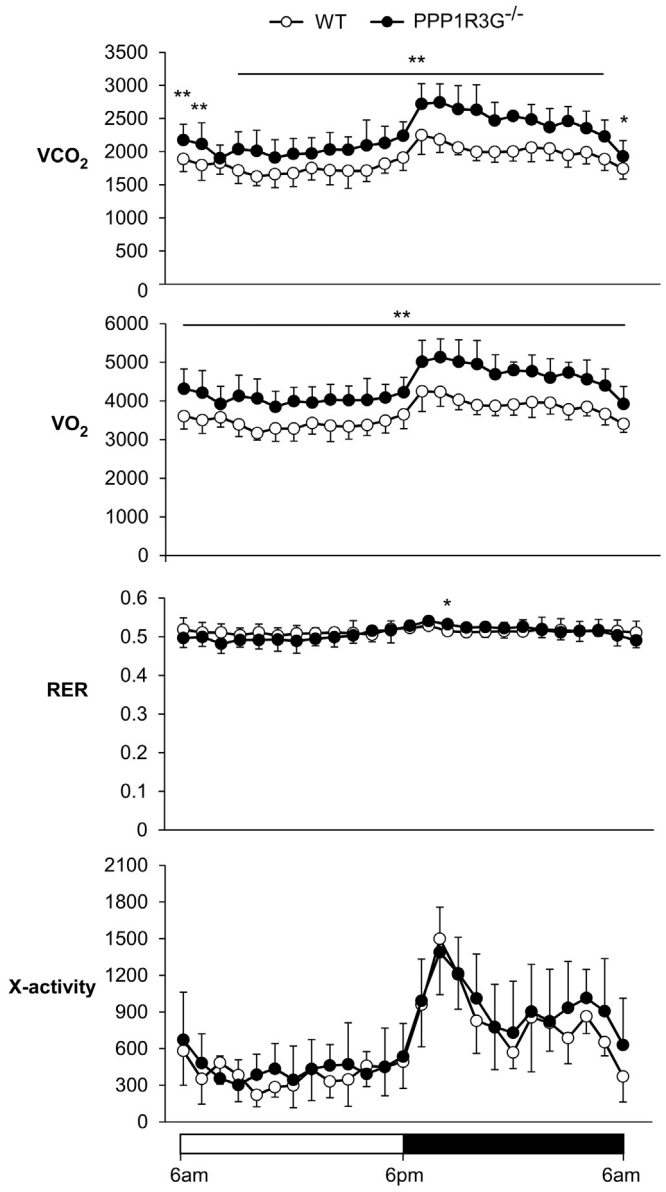

Fig. 3. Metabolic rate is enhanced by PPP1R3G deletion under high-fat diet. The mice as described in Fig. 2 were used to measure their metabolic rate and physical activity through a 12-hr light/dark cycle in a comprehensive laboratory animal-monitoring system. The parameters are shown in an hourly pattern. The data are shown as mean ± SD. * for $P < 0.05$ and ** for $P < 0.01$.

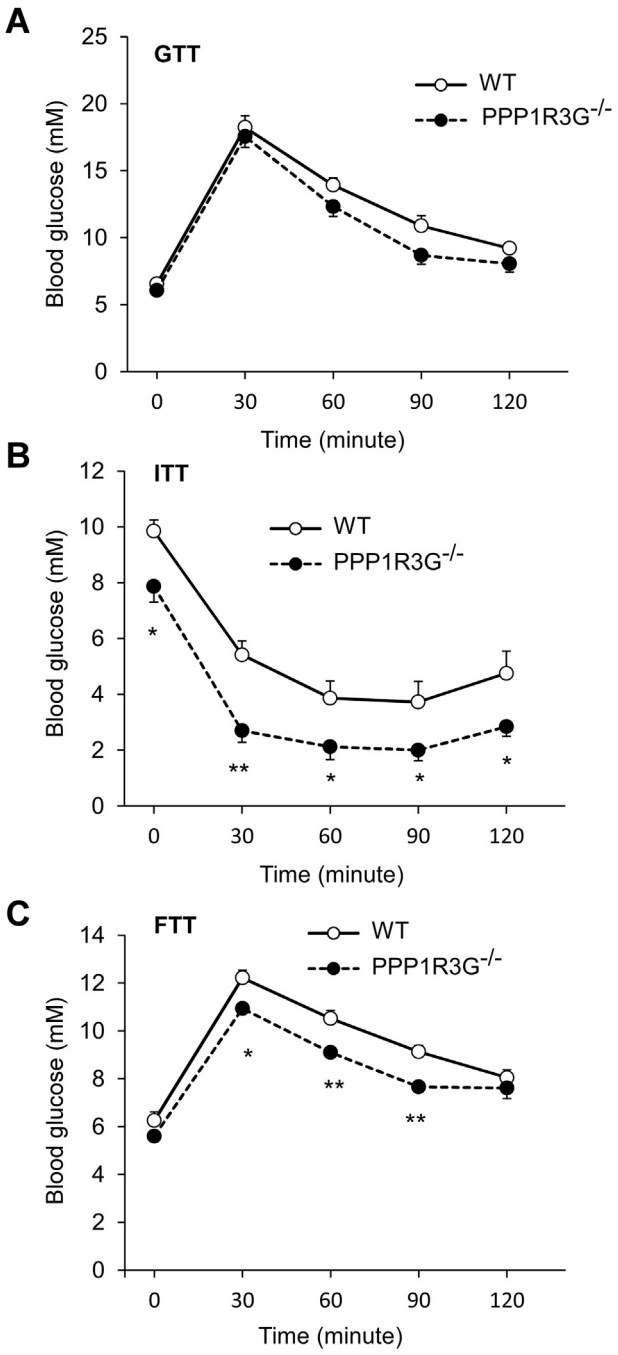

Fig. 4. Deletion of PPP1R3G ameliorates high-fat diet-induced insulin resistance. The mice as described in Fig. 2 were used in glucose tolerance test (A), insulin tolerance test (B), and food tolerance test (C). The data are shown as mean ± SE. * for $P < 0.05$ and ** for $P < 0.01$.

decrease in liver steatosis (Fig. 5) are all secondary to the reduction of obesity in the PPP1R3G$^{-/-}$ mice. These observations are in agreement with numerous other studies that have clearly demonstrated that obesity is the primary cause of alterations in insulin resistance, fatty liver and metabolic rate. It is noteworthy here that glycogen deposition in the liver is clearly associated with hepatic steatosis. Hepatic overexpression of CD36, a fatty acid translocase, is able to promote glycogen synthesis in the mouse liver and reduce high-fat diet induced hepatic steatosis (Garbacz et al., 2016). Such observation is consistent with another study showing that overexpression of PPP1R3G in the mouse liver is capable of increasing glycogen deposition and mitigating fasting-induced hepatic steatosis (Zhang et al., 2014). We postulate that glycogen possesses different effect in different tissues. In the liver, glycogen deposition can reduce triglyceride accumulation and mitigate hepatic steatosis. In the white fat tissue, glycogen is able to promote fat accumulation in adipocytes. The contribution of PPP1R3G to fat deposition in adipose tissue is further supported by our observation that overexpression of PPP1R3G in 3T3L1 cells is able to increase triglyceride level accompanied by an increase in glycogen level (Fig. 6). In conclusion, our study has provided a piece of intriguing evidence not only supporting the notion that glycogen is actively involved in fat accumulation in adipose tissue, but also suggesting that PPP1R3G is an important player in this process. However,

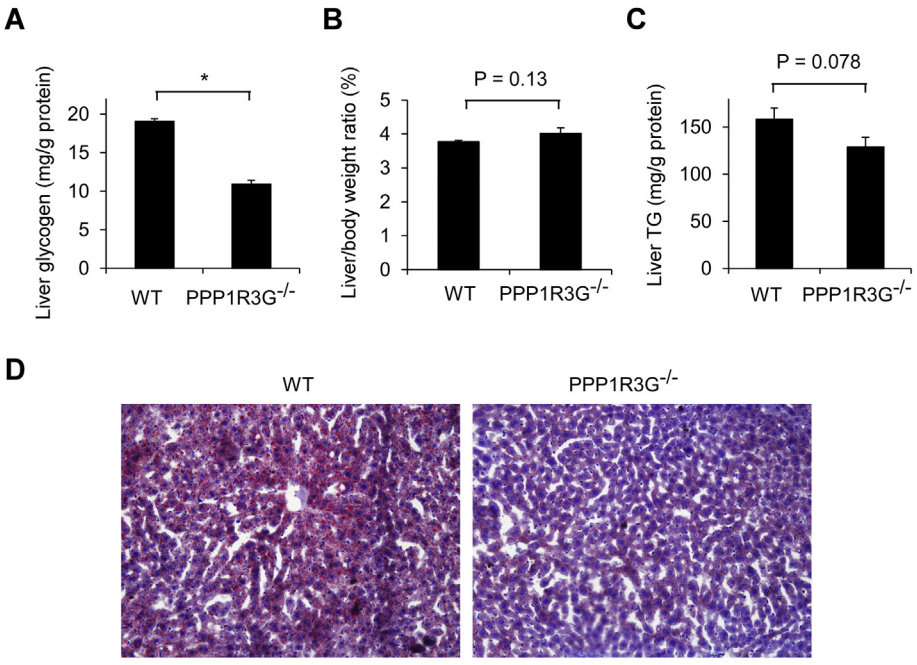

Fig. 5. Deletion of PPP1R3G reduces high-fat diet-induced hepatic steatosis. The mice as described in Fig. 2 were used to measure liver glycogen concentration (A), liver weight (B) and liver triglyceride level (C). The data are shown as mean ± SE. * for $P < 0.05$ and ns for non-significant. Representative images (200 X) of Oil Red O staining are also shown (D).

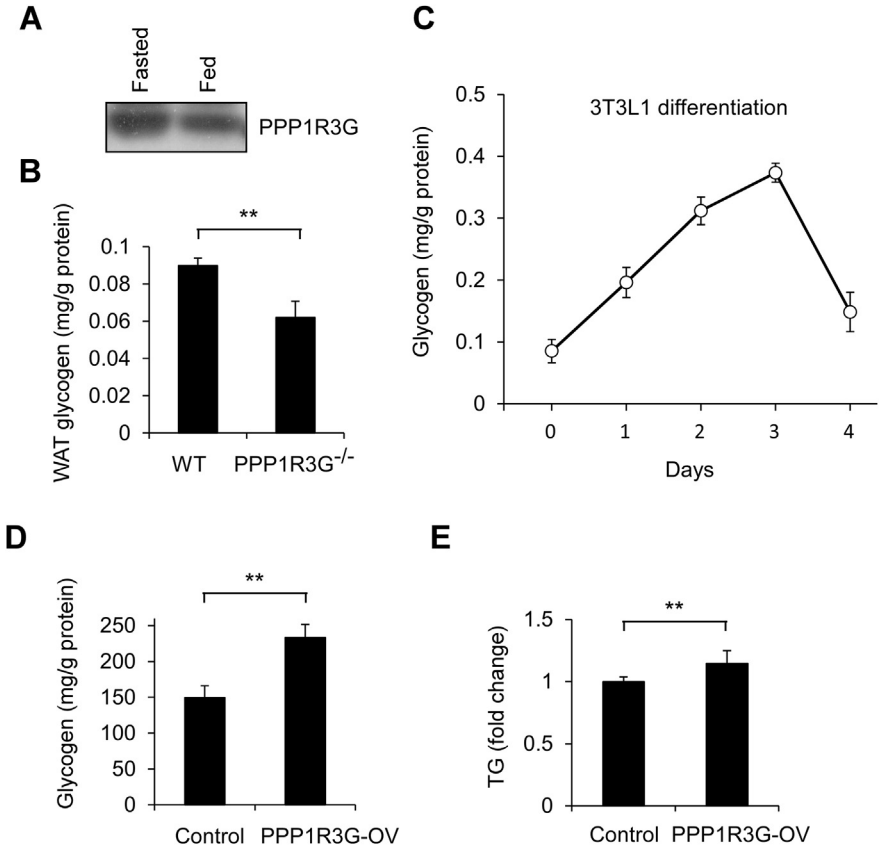

Fig. 6. PPP1R3G affects triglyceride accumulation in adipocytes. (A) PPP1R3G is expressed in WAT. Epididymal WAT under fasting (16 h) or fed condition was used in Western blotting with an antibody against PPP1R3G. (B) The fat glycogen level is reduced by PPP1R3G deletion. The glycogen concentration in the epididymal fat was measured with the mice as described in Fig. 2 under fed condition. (C) The glycogen level changes during the differentiation of 3T3L1 cells. The glycogen concentration was measured during 3T3L1 differentiation at various time point. (D, E) Overexpression of PPP1R3G (PPP1R3G-OV) increases glycogen and triglyceride level in differentiated 3T3L1 cells. The concentrations of glycogen and triglyceride were measured in the cells after differentiation. The data are shown as mean ± SE for A and mean ± SD for C-E, ** for $P < 0.01$.

further determination of the functions of glycogen and PPP1R3G in adipose tissues still awaits a lot more rigorous studies such as using adipocyte-specific deletion PPP1R3G and likely other critical genes involved in glycogen metabolism.

4. Materials and methods

4.1. Animal studies

All animal procedures and protocols were approved by the Institutional Animal Care and Use Committee of the Institute for Nutritional Sciences, Chinese Academy of Sciences. Blood samples were taken and other tissues of interest were snap-frozen in liquid nitrogen immediately after resection and stored at −80 °C until further analysis. Food intake and body weight measurement were described previously (Wang et al., 2013). PPP1R3G-null mice in 129 background were generated by Model Animal Research Center of Nanjing University (Nanjing, China). Tail biopsies of the mice were analyzed by genomic PCR. PPP1R3G-null mice in C57BL/6J background were generated by crossing with C57BL/6J for at least 6 generations. For HFD feeding, 6-week-old male PPP1R3G$^{-/-}$ mice and wild-type littermates were fed with HFD (Research Diets, Inc.) for 20 weeks. Body weight and food intake were monitored weekly. The body fat content was determined by nuclear magnetic resonance (NMR) using a Minispec mq10 nuclear magnetic resonance Analyzer (Bruker Optics). The liver triglyceride level of the mice was measured as previously described (Zhang et al., 2014).

4.2. Immunoblotting analysis

The immunoblotting assay was described in a previous report (Luo et al., 2011). The mouse anti-tubulin antibody was from Sigma-Aldrich (St. Louis, MO, USA). The antibody against PPP1R3G was generated in our laboratory as previously reported (Luo et al., 2011).

4.3. Measurement of glycogen content

The glycogen content of mouse tissues was measured as previously described (Zhang et al., 2014). Measurement of the glycogen content in cells was performed as previously described (Gu et al., 2015).

4.4. Glucose tolerant test (GTT), insulin tolerant test (ITT) and food tolerant test (FTT)

GTT and ITT were performed as previously described (Zhang et al., 2014). For GTT, the mice fasted overnight were injected i.p. with glucose at 1 g/kg. For ITT, the mice fasted for 4–6 h were injected i.p. with insulin at 0.75 unit/kg. For FTT, the mice fasted overnight were fed with normal chow. Blood was collected by venous bleeding from the tail vein at 0, 30, 60, and 120 min after the treatment. Blood glucose concentrations were measured by Glucometer Elite monitor (Abbott Diabetes Care, Alameda, CA, USA).

4.5. Measurement of metabolic rate and physical activity

Metabolic rate and physical activity were determined for animals fed ad libitum using the comprehensive laboratory animal monitoring system (Columbus Instruments, Columbus, OH, USA) according to the manufacturer's instructions. Mice were allowed to acclimate to the system for 16–18 h. Oxygen uptake (VO_2), carbon dioxide production (VCO_2), and the respiratory exchange ratio (RER) were measured in the following 24 h. Voluntary activity was monitored from the x axis beam breaks collected every 20 min.

4.6. Cell differentiation

3T3L1 cells were cultured in high DMEM containing 10% fetal bovine serum (FBS) before the differentiation. At two days post-confluence, the cell culture medium was replaced with the differentiation medium containing 10% FBS, methylisobutylxanthine (115 μg/ml), insulin (1 μg/ml) and dexamethasone (390 ng/ml). In 48 h, the differentiation medium was replaced with medium containing 10% FBS and insulin (1 μg/ml) with a medium change every two days.

4.7. Statistical analysis

The statistical significance was assessed by two-tailed Student's *t*-test. $P < 0.05$ was considered statistically significant.

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References

Agius, L., 2008. Glucokinase and molecular aspects of liver glycogen metabolism. Biochem. J. 414, 1–18.
Birsoy, K., Soukas, A., Torrens, J., Ceccarini, G., Montez, J., Maffei, M., Cohen, P., Fayzikhodjaeva, G., Viale, A., Socci, N.D., Friedman, J.M., 2008. Cellular program controlling the recovery of adipose tissue mass: an in vivo imaging approach. Proc. Natl. Acad. Sci. U. S. A. 105, 12985–12990.
Brady, M.J., Saltiel, A.R., 2001. The role of protein phosphatase-1 in insulin action. Recent Prog. Horm. Res. 56, 157–173.
Ceulemans, H., Bollen, M., 2004. Functional diversity of protein phosphatase-1, a cellular economizer and reset button. Physiol. Rev. 84, 1–39.
Cherrington, A.D., 1999. Banting Lecture 1997. Control of glucose uptake and release by the liver in vivo. Diabetes 48, 1198–1214.
Crosson, S.M., Khan, A., Printen, J., Pessin, J.E., Saltiel, A.R., 2003. PTG gene deletion causes impaired glycogen synthesis and developmental insulin resistance. J. Clin. Investig. 111, 1423–1432.
Delibegovic, M., Armstrong, C.G., Dobbie, L., Watt, P.W., Smith, A.J., Cohen, P.T., 2003. Disruption of the striated muscle glycogen targeting subunit PPP1R3A of protein phosphatase 1 leads to increased weight gain, fat deposition, and development of insulin resistance. Diabetes 52, 596–604.
Garbacz, W.G., Lu, P., Miller, T.M., Poloyac, S.M., Eyre, N.S., Mayrhofer, G., Xu, M., Ren, S., Xie, W., 2016. Hepatic overexpression of CD36 improves glycogen homeostasis and attenuates high-fat diet induced hepatic steatosis and insulin resistance. Mol. Cell Biol. 36, 2715–2727 (online).
Gu, J., Zhang, Y., Xu, D., Zhao, Z., Zhang, Y., Pan, Y., Cao, P., Wang, Z., Chen, Y., 2015. Ethanol-induced hepatic steatosis is modulated by glycogen level in the liver. J. Lipid Res. 56, 1329–1339.
Hughes, K., Nikolakaki, E., Plyte, S.E., Totty, N.F., Woodgett, J.R., 1993. Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. EMBO J. 12, 803–808.
Jurczak, M.J., Danos, A.M., Rehrmann, V.R., Allison, M.B., Greenberg, C.C., Brady, M.J., 2007. Transgenic overexpression of protein targeting to glycogen markedly increases adipocytic glycogen storage in mice. Am. J. Physiol. Endocrinol. Metab. 292, E952–E963.
Kelsall, I.R., Rosenzweig, D., Cohen, P.T., 2009. Disruption of the allosteric phosphorylase a regulation of the hepatic glycogen-targeted protein phosphatase 1 improves glucose tolerance in vivo. Cell Signal 21, 1123–1134.
Kelsall, I.R., Voss, M., Munro, S., Cuthbertson, D.J., Cohen, P.T., 2011. R3F, a novel membrane-associated glycogen targeting subunit of protein phosphatase 1 regulates glycogen synthase in astrocytoma cells in response to glucose and extracellular signals. J. Neurochem. 118, 596–610.
Luo, X., Zhang, Y., Ruan, X., Jiang, X., Zhu, L., Wang, X., Ding, Q., Liu, W., Pan, Y., Wang, Z., Chen, Y., 2011. Fasting-induced protein phosphatase 1 regulatory subunit contributes to postprandial blood glucose homeostasis via regulation of hepatic glycogenesis. Diabetes 60, 1435–1445.
Markan, K.R., Jurczak, M.J., Brady, M.J., 2010. Stranger in a strange land: roles of glycogen turnover in adipose tissue metabolism. Mol. Cell Endocrinol. 318, 54–60.
Munro, S., Ceulemans, H., Bollen, M., Diplexcito, J., Cohen, P.T.W., 2005. A novel glycogen-targeting subunit of protein phosphatase 1 that is regulated by insulin and shows differential tissue distribution in humans and rodents. Febs J. 272, 1478–1489.

Nuttall, F.Q., Gilboe, D.P., Gannon, M.C., Niewoehner, C.B., Tan, A.W., 1988. Regulation of glycogen synthesis in the liver. Am. J. Med. 85, 77–85.
Printen, J.A., Brady, M.J., Saltiel, A.R., 1997. PTG, a protein phosphatase 1-binding protein with a role in glycogen metabolism. Science 275, 1475–1478.
Rubio-Villena, C., Garcia-Gimeno, M.A., Sanz, P., 2013. Glycogenic activity of R6, a protein phosphatase 1 regulatory subunit, is modulated by the laforin-malin complex. Int. J. Biochem. Cell Biol. 45, 1479–1488.
Shulman, R.G., Rothman, D.L., 1996. Enzymatic phosphorylation of muscle glycogen synthase: a mechanism for maintenance of metabolic homeostasis. Proc. Natl. Acad. Sci. U. S. A. 93, 7491–7495.
Tuerkischer, E., Wertheimer, E., 1942. Glycogen and adipose tissue. J. Physiol. 100, 385–409.
Villarpalasi, C., 1991. Substrate specific activation by glucose-6-phosphate of the dephosphorylation of muscle glycogen-synthase. Biochim. Biophys. Acta 1095, 261–267.
Wang, L., Wang, X., Li, Z., Xia, T., Zhu, L., Liu, B., Zhang, Y., Xiao, F., Pan, Y., Liu, Y., Guo, F., Chen, Y., 2013. PAQR3 has modulatory roles in obesity, energy metabolism, and leptin signaling. Endocrinology 154, 4525–4535.
Zhang, Y., Xu, D., Huang, H., Chen, S., Wang, L., Zhu, L., Jiang, X., Ruan, X., Luo, X., Cao, P., Liu, W., Pan, Y., Wang, Z., Chen, Y., 2014. Regulation of glucose homeostasis and lipid metabolism by PPP1R3G-mediated hepatic glycogenesis. Mol. Endocrinol. 28, 116–126.