Liver glycogen reduces food intake and attenuates obesity in a high-fat diet-fed mouse model

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Abstract

We generated mice that overexpress protein targeting to glycogen (PTG) in the liver \((\text{PTG}^{\text{OE}})\), which results in an increase in liver glycogen. When fed a high-fat diet \((\text{HFD})\), these animals reduced their food intake. The resulting effect was a lower body weight, decreased fat mass and reduced leptin levels. Furthermore, PTG overexpression reversed the glucose intolerance and hyperinsulinemia caused by the HFD and protected against HFD-induced hepatic steatosis. Remarkably, when fed a HFD, PTG\(^{\text{OE}}\) mice did not show the decrease in hepatic ATP content observed in control animals and had lower expression of neuropeptide Y (NPY) and higher expression of propiomelanocortin (POMC) in the hypothalamus. Additionally, after an overnight fast, PTG\(^{\text{OE}}\) animals presented high liver glycogen content, lower liver triacylglycerol content, and lower serum concentrations of fatty acids and \(\beta\)-hydroxybutyrate compared to control mice, regardless whether they received a HFD or a standard diet (SD). In conclusion, liver glycogen accumulation caused a reduced food intake, protected against the deleterious effects of a HFD and diminished the metabolic impact of fasting. Therefore, we propose that hepatic glycogen content be considered a potential target for the pharmacological manipulation of diabetes and obesity.
Liver glycogen acts as an energy store in times of nutritional sufficiency for use in times of need. The metabolism of this polysaccharide in the liver is controlled by the activities of two key enzymes: glycogen synthase (GS) and glycogen phosphorylase (GP) (1). GS is phosphorylated at multiple sites, which induces its inactivation while GP is activated by phosphorylation at a single site. Both enzymes are also regulated allosterically (2; 3).

Glycogen targeting subunits (G subunits) bind to glycogen and to protein phosphatase 1 (PP1) and facilitate the dephosphorylation of GS and GP, thus activating the former and inactivating the latter. There are six genes encoding G subunits (4). Among these, PTG (PP1R3C or PPP1R5), which is expressed in many tissues, has been shown to control glycogen stores in various animal models (5-7).

Adenoviral PTG overexpression in the liver of normal rats increased glycogen and improved glucose tolerance without perturbing lipid metabolism (8). In a diabetic-focused approach, Yang et al. (9) showed that adenoviral expression of PTG in the liver of STZ-diabetic rats increased glycogen content and reversed hyperglycemia and hyperphagia. Through a different approach, we reported that hepatic adenoviral expression of an active form of liver glycogen synthase (LGS), which also increased glycogen content, in STZ-diabetic rats reduced food intake and hyperglycemia (10).

Russek was the first to propose a “hepatostatic” theory of food intake (11), further redefined as a “glycogenostatic” model by Flatt (12). This model predicts that individuals consume food to a level that maintains glycogen levels in the body (12). In fact, many lines of experimental evidence establish a correlation between the size of liver glycogen stores and food intake (13; 14); however, other results argue against this hypothesis (15-17). The results in (9) and (10) support the notion that liver glycogen is a factor controlling food intake in hyperphagic type 1 diabetic animals. However, these studies had the constraint of using adenoviral-transduced animals, which limits the
experimental period to one week. Here we examined whether a sustained increase in liver glycogen stores regulates food intake. For this purpose, we generated mice that overexpress PTG specifically in the liver (PTG\textsuperscript{OE}), which results in a sustained increase in hepatic glycogen, and fed them a standard (SD) or a high-fat (HFD) diet. The HFD-fed animal is a suitable model for studying impaired glucose tolerance and type 2 diabetes (18), and prolonged ingestion of a HFD is associated with overconsumption and obesity (19).

Here we show that when fed a HFD, PTG\textsuperscript{OE} animals reduced their food intake and presented a lower body weight and fat mass than controls. These results identify liver glycogen stores as a regulator of food intake in a model of hyperphagia and obesity, and suggest that strategies to increase liver glycogen may provide a treatment option for diabetes and obesity.
RESEARCH DESIGN AND METHODS

Mice

To generate mice that overexpress PTG, targeting-vector construction and a site-directed transgenic strategy were designed and performed by genOway (Lyon, France). Briefly, the PTG cDNA under the control of the ubiquitous CAG promoter (CMV immediate early enhancer/chicken β-actin promoter fusion) was introduced into an innocuous locus by homologous recombination. A loxP-flanked transcription stop cassette was included between the CAG promoter and the PTG cDNA to allow the expression to be dependent upon the action of a Cre recombinase. The resulting mouse line was bred with an albumin promoter Cre recombinase-expressing animal (The Jackson Laboratory), which drove the expression of PTG specifically to the liver. All mice studied were littermates. Mice were maintained on a 12:12 h light-dark cycle with free access to water and fed a SD (Harlan Research Laboratories) or HFD (45% Kcal fat; Catalogue # D12451 Research Diets) for 16 weeks (starting at 6 weeks of age).

Blood and liver biochemical analysis

Liver glycogen content was determined by an amylglucosidase-based assay, as described elsewhere (20). Liver glycogen synthase activity was determined as previously described in the presence or absence of Glc-6-P (10). GS activity measured in the presence of saturating Glc-6-P ((+) Glc-6-P) corresponds to the total amount of enzyme, whereas measurement in its absence ((-) Glc-6-P) is an indication of the active (unphosphorylated) GS form. The (-) Glc-6-P /(+) Glc-6-P activity ratio (GS activity ratio) is an estimation of the activation state of the enzyme. The intracellular concentration of ATP was measured from perchloric acid extracts of livers using a fluorimetric method, as described previously (21). Triglycerides in liver were quantified using a TAG kit (Sigma), in 3 mol/l KOH, 65% ethanol extracts, based on the method
described by Salmon and Flatt for liver saponification (22). Blood glucose levels were measured using a glucometer (Ascensia Breeze 2, Bayer Healthcare). Serum concentrations of β-hydroxybutyrate (Sigma), triacylglycerides (Sigma) and non-esterified fatty acids (Wako) were measured spectrophotometrically. Plasma insulin and leptin were analyzed by ELISA (Crystal Chem).

**Glucose and insulin tolerance tests**

For glucose tolerance tests, overnight fasted (16 h) mice were injected i.p. with glucose (2 g/kg). Whole blood was drawn from tail tip for glucose measurements. In vivo glucose-stimulated insulin secretion was determined in separate experiments. For insulin tolerance tests, mice fasted for 6 h were injected i.p. with insulin (0.75 U/kg), and glycemia was measured from tail blood taken at the indicated times after injection.

**RNA preparations and quantitative RT-PCR**

Tissue preparation, RNA extraction, RT-PCR, and quantitative RT-PCR analyses were performed as described (23). The following TaqMan primer/probe sets (Applied Biosystems, Madrid, Spain) were used for qRT-PCR: PTG (Mm01204084_m1), Hprt (Mm00446968_m1), Pklr (Mm00443090_m1), Fasn (Mm00662319_m1), Acc1α (Mm01304257_m1), SREBP1 (Mm00550338_m1), PPARγ (Mm01184322_g1), MGAT1 (Mm00503358_m1), GK (Mm00439129), Ucp1 (Mm01244861_m1), Ucp2 (Mm00495907_g1), Ucp3 (Mm00494077_m1), PPARα (Mm00440939_m1), Fgf21 (Mm00840165_g1), NPY (Mm03048253), POMC (Mm 00435874_m1), Cpt1α (Mm01231183_m1), Acox1 (Mm 01246834_m1) and Ppia (Mm02342429_g1). Ppia was used as housekeeping gene in the liver. Hprt was used as housekeeping gene in the hypothalamus and brown adipose tissue.
**Indirect calorimetry, food intake, and body temperature**

Indirect calorimetry was performed using an 8-chamber Oxymax system (Columbus Instruments) to measure heat production, calculated from oxygen consumption and CO₂ production. Mice were allowed to acclimate to the cages for 2 days before 1 or 2 cycles of 24-h measurements. Energy expenditure was calculated as $EE = (3.185 + 1.232 \times RER) \times V_O_2 \ (24)$ and respiratory exchange ratio (RER) as $RER = \frac{V_{CO_2}}{V_{O_2}}$. Glucose oxidation (in g/min/kg$^{0.75}$ = $\frac{[(4.545 \times V_{CO_2}) - (3.205 \times V_{O_2})]}{1000}$), and lipid oxidation (in g/min/kg$^{0.75}$ = $\frac{[1.672 \times (V_{O_2} - V_{CO_2})]}{1000}$) were calculated. Ambulatory and total locomotor activity was monitored by an infrared photocell beam interruption method. Body temperature was determined using an animal rectal probe thermometer (Cibertec).

In order to monitor food intake mice were housed individually and acclimatized for 1 week prior to study. Food intake was measured daily for 5 consecutive days. Epididymal adipose tissue was removed and prepared in paraffin after fixation in 10% phosphate-buffered formalin. Hematoxylin and eosin (H&E) stains were then performed. To measure the size of the adipocytes, total adipocyte area was traced manually and analyzed. White adipocyte areas were measured in 100 or more cells per mouse in each group.

**Oil red O staining**

Liver tissues were embedded in OCT compound (Sakura Finetek) and frozen. Frozen tissues were cut into 5-μm thick cryosections and stained with Oil Red O (Sigma).

**Statistics**

Data are expressed as mean ± SEM. p values were calculated using unpaired Student’s t test, two-way ANOVA or three-way ANOVA with post hoc Tukey tests as appropriate. p < 0.05 was considered significant.
Study approval

All procedures were approved by Barcelona Science Park’s Animal Experimentation Committee and were carried out in accordance with the European Community Council Directive and the National Institute of Health guidelines for the care and use of laboratory animals.
RESULTS

Generation and characterization of mice with liver-specific PTG overexpression

To increase the accumulation of glycogen in the liver, we generated mice that overexpress PTG specifically in the liver (PTG^{OE}) (see Methods). The mRNA level of PTG in the livers of these mice was 12-fold greater than that of control animals (Figure 1A). As expected, LGS was activated since PTG, by targeting PP1 to the glycogen particle, maintains glycogen synthase and glycogen phosphorylase in a dephosphorylated state (7). As expected, LGS was activated in these mice (Figure 1B).

Mice were fed a SD or HFD. When fed, the PTG^{OE} mice showed a ~2-fold increase in liver glycogen content compared to control animals (Figure 1C), regardless of whether they received a SD or HFD (Figure 1C).

After an overnight fast, PTG^{OE} mice showed decreased hepatic glycogen content compared with the fed state. This observation indicates net mobilization of liver glycogen, although this glycogen was not depleted to the same extent as in control mice (Figure 1C). Moreover, when fasted, the control mice receiving the HFD showed a higher hepatic glycogen content than SD-fasted control animals, as previously reported (25) (Figure 1C).

Skeletal muscle glycogen content was similar between the groups and thus consistent with the idea that glycogen synthesis in non-hepatic tissues was not altered (Figure 1D).

HFD-fed PTG^{OE} mice have a lower food intake and reduced obesity

Mice fed a HFD present hyperphagia and obesity (26; 27). In our experiments, mice of both genotypes fed a SD had similar body weights (Figure 2A). Those on a HFD became obese compared to counterparts fed a SD (Figure 2A). However, the increase in body weight in PTG^{OE} animals, when fed a HFD, was smaller than that detected in
control mice on the same diet (Figure 2A). After 16-week on a HFD, PTG\textsuperscript{OE} animals showed a reduction in epididymal and subcutaneous fat weight, while when fed a SD they had a similar fat weight as their control littermates (Figure 2B and 2C). Control animals on a HFD had larger adipocytes than PTG\textsuperscript{OE} mice on the same diet (Figure 2D). Consistent with the reduced adiposity of PTG\textsuperscript{OE} mice fed a HFD, serum leptin concentration was significantly lower in these animals compared to control mice on the same diet, while no differences in serum leptin were found between the two genotypes when fed a SD (Figure 2E). The decreased adiposity and reduced HFD-induced obesity could result from reduced food intake, increased energy expenditure, or fat malabsorption. PTG\textsuperscript{OE} mice on a HFD showed a reduced daily food intake of around 20% compared to control mice fed the same diet (Figure 2F). The hypothalamus is the master regulator of energy intake, energy expenditure, and body weight (28). In the hypothalamus of PTG\textsuperscript{OE} fed a HFD, the expression of the satiating neuropeptide POMC was higher, while that of the orexigenic peptide NPY was lower than in control mice fed the same diet (Figure 2G).

We also determined the total daily energy expenditure in the two genotypes by measuring \( V_{\text{O}_2} \) and \( V_{\text{CO}_2} \). When fed a SD, control and PTG\textsuperscript{OE} mice had a similar oxygen consumption (Figure 3A), energy expenditure (standardized for body weight) (Figure 3C), locomotor activity (Figure 3E and 3G), and respiratory exchange ratio (RER) (Figure 4A). No significant difference in oxygen consumption (Figure 4B), energy expenditure (Figure 4D), or locomotor activity (Figure 3B and 3D) was observed between the HFD-fed groups. In order to examine the influence of liver glycogen content on thermogenesis, mRNA expression of Ucp1, a marker of thermogenesis, was analyzed in brown adipose tissue. No difference was found in the levels of Ucp1 mRNA between control and PTG\textsuperscript{OE} mice fed a HFD (Figure 4G). Other genes involved in
energy expenditure, such as Ucp2 and Ucp3, were also analyzed, and no differences were found between the groups (Figure 4G). No changes in core body temperature were detected in the two genotypes (Figure 4H). Moreover, there were no differences in stool lipid content (data not shown).

During the feeding period (dark phase), the RER was slightly increased in HFD-PTG\textsuperscript{OE} mice, thereby indicating that these animals used more carbohydrates as an energy source than the control group at night (Figure 4B). These results were confirmed by calculating the amount of glucose oxidized, which was increased in PTG\textsuperscript{OE} mice fed a HFD (Figure 4D). However, PTG\textsuperscript{OE} fed a SD oxidized the same amount of glucose as control mice (Figure 4C). No change in lipid oxidation was found in PTG\textsuperscript{OE} mice fed a SD (Figure 4E) or HFD (Figure 4F). Since glucose oxidation was higher in PTG\textsuperscript{OE} fed a HFD, we addressed hepatic ATP content. It is known that this parameter is reduced in the livers of HFD-induced diabetic mice (29). The ATP content in the liver of HFD-fed mice was significantly reduced, and PTG overexpression resulted in an ATP content similar to that of mice fed a SD (Figure 2H).

**Effects of liver PTG overexpression on blood glucose, insulin levels, glucose tolerance, and insulin sensitivity**

The liver plays a key role in the clearance of blood glucose in the postpandrial state (30). Fed PTG\textsuperscript{OE} animals had lower blood glucose levels than control littermates, regardless of the diet received (Figure 5A). Blood glucose levels and plasma insulin concentration decreased in control animals when they were deprived of food for 16 h (Figure 5A and 5B). However, fasted PTG\textsuperscript{OE} animals had similar glucose and insulin levels as fed PTG\textsuperscript{OE} mice. This effect was observed regardless of the diet given (Figure...
Moreover, when fed a HFD, PTG\textsuperscript{OE} mice showed lower levels of insulin in the fed state compared to control mice on the same diet (Figure 5B).

We next performed an i.p. glucose tolerance test (IPGTT) on all four experimental groups. PTG\textsuperscript{OE} animals fed a SD displayed better glucose tolerance, with a 40% decrease in the AUC compared to control mice. When subjected to a HFD, these mice also showed reduced glucose intolerance and presented a 25% decrease in the AUC compared to control littermates (Figure 5C). Remarkably, when fed a HFD, PTG\textsuperscript{OE} mice presented a glucose tolerance curve analogous to that of control animals fed a SD (Figure 5C).

We also measured insulin secretion during the IPGTT. In response to a HFD, PTG\textsuperscript{OE} animals presented a reduction in glucose-stimulated insulin release compared to control mice (Figure 5D). Of note, the insulin release in the former animals was similar to that of PTG\textsuperscript{OE} animals on a SD (Figure 5D).

Next, an insulin tolerance test (ITT) was performed after a 6-h fast. In these conditions, PTG\textsuperscript{OE} animals on a HFD already had significantly lower blood glucose concentration than the HFD-control group (14±1.7 versus 10±0.3 mM), which made the analysis of the results of the ITT difficult to compare (Figure 5E). However, when the ITT was expressed as the percentage of the initial values, the curve for HFD animals was similar in both genotypes (Figure 5F). Remarkably, PTG\textsuperscript{OE} mice on a SD presented higher blood glucose levels 60 min after the insulin injection. This finding suggests that these animals showed a faster recovery from the hypoglycemia induced by insulin than control littermates (Figure 5E and 5F).
Liver PTG overexpression reduces HFD-induced hepatic steatosis

We also analyzed the effect of PTG overexpression on the storage of liver triacylglycerides. When fed a SD, PTG\textsuperscript{OE} mice presented a similar liver triacylglycerol content as their control littermates (Figure 6A and 6B), suggesting that PTG is not associated with lipid metabolism under these circumstances. Moreover, the expression of genes related to de novo lipogenesis were not modified in PTG\textsuperscript{OE} animals fed a SD (Figure 6C). However, when fed a HFD, these animals showed a lower hepatic triglyceride content (Figure 6A and 6B), which was associated with the downregulation of SREBP1, glucokinase (GK), PPAR\textgreek{y}, and monoacylglycerol O-acyltransferase1 (MGAT1) gene expression (Figure 6C and 6D). We confirmed that, as previously described (31), PPAR\textgreek{y} and MGAT1 expression was very low in normal liver but was highly express in the fatty liver (Figure 6D). There were no statistically significant differences in the expression of lipogenic genes, such as pyruvate kinase (Pklr), fatty acid synthase (Fasn), and acetyl coA carboxylase (Acc1\textalpha), between HFD-fed groups (Figure 6C). Furthermore, the expression of genes related to lipid oxidation was evaluated. No differences between genotypes were found in the expression of PPAR\textgreek{a}, carnitine palmitoyltransferase 1 (Cpt1\textalpha) or acyl-coA oxidase (Acox) in the liver (Figure 6E).

Liver PTG overexpression diminishes the metabolic impact of fasting

Many metabolic changes take place during fasting. As mentioned above, PTG\textsuperscript{OE} mice fed either a SD or HFD did not show reduced levels of blood glucose or insulin after an overnight fast (Figure 5A, 5B). Moreover, serum non-esterified fatty acids (Figure 7A) and \textbeta-hydroxybutyrate (Figure 7B) were lower in PTG\textsuperscript{OE} fasted animals. It is well known that after an overnight fast hepatic triacylglycerol content increases in mice (32; 33). However, PTG\textsuperscript{OE} animals presented a lower fasting liver triacylglycerol content.
compared to control mice (Figure 7C and 7D). During fasting the expression of the hepatokine Fgf21 increased in the liver, but no differences were found in the expression of Fgf21 between genotypes (data not shown).
DISCUSSION

Using mice that overexpress PTG specifically in the liver, here we examined the impact of liver glycogen on food intake. The overexpression of this protein caused the mice to increase their hepatic glycogen stores. When fed a HFD, these animals decreased their food intake and had a lower body weight and decreased fat mass. Changes in key regulators of food intake in the hypothalamus support the decrease in appetite observed in those animals. Expression of POMC, an anorexigenic signal increased, while that of orexigenic NPY decreased. These data support the idea that liver glycogen stores regulate food intake, thus reinforcing the glycogenostatic theory (12). However, in our case, this effect was limited to hyperphagic conditions, such as HFD. Friedman proposed that changes in glycogen stores do not necessarily signal changes in food intake, but rather that the partitioning of carbohydrates in and out of glycogen affects eating behavior by altering fuel fluxes (34) and that, by analogy to fat fuels, shifts between oxidation and storage of carbohydrate fuels influence food intake (34). PTG\textsuperscript{OE} mice used more carbohydrates as an energy source than control animals during the dark phase, which is when mice typically eat more. This increase in carbohydrate oxidation was observed in animals on a HFD, but not when on a SD. These data highlight the role of liver glycogen stores in modulating energy substrate utilization in response to a HFD.

ATP is a final product of the oxidation of glucose and fatty acids. It has been proposed that a decrease in the amount of hepatic ATP is a metabolic stimulus that triggers feeding behavior (35; 36). We found that consumption of a HFD decreased hepatic ATP levels. Remarkably, PTG\textsuperscript{OE} mice maintained hepatic ATP content when fed a HFD. We propose that increased liver glycogen stores, through the maintenance of liver energy status, contribute to decreased appetite and adiposity. This effect is probably triggered...
by signals from the liver that are carried to the brain by vagal sensory neurons, as previously reported (37; 38).

In addition, PTG\textsuperscript{OE} mice on a SD showed improved glucose tolerance. Consistent with this notion, overexpression of PTG induced by adenovirus in normal rats resulted in a modest improvement of glucose tolerance; however, these animals failed to lower glycogen levels in response to fasting (8). In our model of liver PTG-overexpressing mice, animals degraded glycogen in response to fasting, although they were not able to completely deplete the stores of this polysaccharide after a 16 h fasting period. More importantly, PTG overexpression reversed HFD-induced glucose intolerance and hyperinsulinemia. Similar studies showed that expression using adenovirus-of other targeting subunit isoforms of PP1, such as the truncated version of muscle isoform termed G\textsubscript{MΔC}, ameliorated glucose intolerance in rats on a HFD, but did not reduce the high fasting insulin levels of these animals (39).

Also noteworthy was the effect of PTG overexpression in decreasing hepatic steatosis induced by HFD. The reduction in feeding observed in PTG\textsuperscript{OE} mice on a HFD may account for the lower levels of hepatic triacylglycerol in the fed condition. This decrease in hepatic steatosis was associated with a decrease in the expression of PPAR\textgreek{gamma} and MGAT1. PPAR\textgreek{gamma} is a transcriptional factor that participates in hepatic steatosis in rodents (40; 41), and it has recently been described that PPAR\textgreek{gamma}-regulated MGAT1 expression is responsible for lipid accumulation in diet-induced hepatic steatosis (31). SREBP1 and GK expression were downregulated in the HFD-PTG\textsuperscript{OE} group. SREBP1 and GK expression is stimulated by insulin (42-44), and both genes contribute to hepatic steatosis (45; 46). Since PTG overexpression reversed HFD-induced hyperinsulinemia, the lower insulin levels in PTG\textsuperscript{OE} mice may account for the
downregulation of SREBP1 and GK. The expression of some of the main lipogenic genes regulated by SREBP1, such as Acc1α and Fasn (47), as well as genes related to lipid oxidation was not statistically different between the genotypes. We therefore conclude that neither changes in de novo lipogenesis nor in lipid oxidation contribute to the decrease in hepatic lipid content in the HFD-fed PTG^{OE} mouse group.

A recent study reveals that glycogen shortage in the liver triggers the liver-brain-adipose neural axis independently of glucose and insulin/glucagon levels, thus having a key role in switching the fuel source from glycogen to triglycerides under prolonged fasting conditions (48). Our results support these observations, since overnight fasted PTG^{OE} mice had higher glycogen levels than control animals. Consequently, many of the changes in metabolism that occur during fasting when the glycogen supply dwindles were attenuated in these animals. PTG^{OE} mice showed a markedly lower hepatic lipid content during fasting. This could be attributable to a lower flux of fatty acids arriving from adipose tissue to the liver. These animals also showed lower levels of fatty acids and β-hydroxybutyrate in serum under fasting conditions, suggesting that lipolysis in adipose tissue is attenuated, as is ketogenesis in the liver. While the impact of increased PTG or increased hepatic glycogen is consistent throughout the literature, the effects of decreased PTG appear to be more complex. In a first report (49), it was shown that heterozygous deletion of PTG in mice reduced glycogen levels and induced glucose intolerance and insulin resistance with age. These observations are in agreement with other studies of animal models where glycogen stores are decreased by other means, such as depletion of liver glycogen synthase (50). However, in a recent paper (25), it has been reported that total ablation of PTG reduced fasting glucose and insulin levels in obese mice, while improving insulin sensitivity and blocking hepatic steatosis during fasting in HFD-fed mice. Changes in the background of the animals used may explain
these differences and may indicate that the consequences of decreased PTG are greatly influenced by other factors. It is also important to stress that in that study (25), homozygous deletion of PTG is constitutive, and the effects caused by PTG depletion are not circumscribing to the liver. Therefore PTG depletion in other tissues, such as adipose tissue, may affect the whole picture. In contrast, we have generated mice with a liver-specific overexpression of PTG to avoid interferences with the effect of PTG in other tissues.

Our results demonstrate that liver glycogen accumulation prevents HFD-induced glucose intolerance, decreases food intake, and lowers body weight. In conclusion, our results point to hepatic glycogen content as a potential target for the pharmacological manipulation of diabetes and obesity.
Author contribution: ILS, DZ, AA researched data. ILS, JIG wrote the manuscript. JD designed the PTG overexpressing mouse. ILS, DZ, JC, JIG designed the study. ILS, JD, DZ, AA, JIG and JC revised the manuscript. JIG is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The authors declare that they have no conflict of interest in relation to this work.

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FIGURE LEGENDS:

Figure 1. Characterization of mice with liver-specific PTG overexpression fed a SD or HFD. Control mice and liver PTG$^{OE}$ mice aged 6 weeks were put on a SD or HFD for 16 weeks. Fed or 16-h fasted mice were sacrificed. (A) Relative mRNA of PTG in the liver under fed conditions. (B) Liver glycogen synthase (GS) activity expressed as the ratio (-G6P/+G6P) under fed conditions. (C) Liver glycogen content under fed conditions or a 16-h fast. (D) Quadricep muscle glycogen content under fed conditions. Data are mean ± SEM. n=5–8/per group; * P<0.05 between control mice and PTG$^{OE}$ mice fed a SD or HFD, ^ P<0.05 between control fed mice and control 16-h fasted mice fed a SD or HFD, # P<0.05 between fed PTG$^{OE}$ mice and fasted PTG$^{OE}$ mice. § P<0.05 between control fasted mice fed a SD and control fasted mice fed a HFD.

Figure 2. HFD-fed PTG$^{OE}$ mice have a lower food intake and reduced obesity. Control mice and liver PTG$^{OE}$ mice aged 6 weeks were put on a SD or HFD for 16 weeks. Fed or 16-h fasted mice were sacrificed. (A) Growth curve. Body weights were measured every other week. (B) Epididymal adipose tissue. (C) Subcutaneous adipose tissue. (D) H&E from epididymal adipose tissue, scale bar 200µm and adipocyte size from epididymal adipose tissue. (E) Fasting serum leptin. (F) Food intake. (G) Quantitative real-time PCR showing relative mRNA level of POMC and NPY in the hypothalamus of mice fed a HFD. (H) Relative liver ATP content of mice fed a SD or HFD. Data are mean ± SEM. n=5–20/per group.* P<0.05 between control mice and PTG$^{OE}$ mice fed a SD or HFD. # P<0.05 between SD and HFD.

Figure 3. Energy expenditure in control and PTG overexpressing animals fed a SD or HFD. Control mice and liver PTG$^{OE}$ mice aged 6 weeks were put on a SD or HFD for 16 weeks. (A) Resting oxygen consumption during the light phase, dark phase and
total of animals on SD. (B) Resting oxygen consumption during the light phase, dark phase and total of animals on a HFD. (C) Resting energy expenditure during the light phase, dark phase and total of animals on a SD. (D) Resting energy expenditure during the light phase, dark phase and total of animals on HFD. (E) Locomotor activity (ambulation) during light phase, dark phase, and total activity of animals on a SD. (F) Locomotor activity (ambulation) during light phase, dark phase, and total activity of animals on HFD. (G) Locomotor activity (total counts) during light phase, dark phase, and total activity of animals on a SD. (H) Locomotor activity (total counts) during the light phase, dark phase, and total activity of animals on a HFD. Data are mean ± SEM. n=6–8/per group.* P<0.05 between control mice and PTG^{OE} mice fed a SD or HFD.

Figure 4. Respiratory exchange ratio, glucose and lipid oxidation. Control mice and liver PTG^{OE} mice aged 6 weeks were put on a SD or HFD for 16 weeks. (A) Respiratory exchange ratio (RER) during the light phase, dark phase and total of animals on a SD. (B) Respiratory exchange ratio (RER) during the light phase, dark phase, and total of animals on HFD. (C) Glucose oxidation of animals on a SD during the light phase, dark phase, and total. (D) Glucose oxidation of animals on a HFD during the light phase, dark phase, and total. (E) Lipid oxidation of animals on a SD during the light phase, dark phase, and total. (F) Lipid oxidation of animals on a HFD during the light phase, dark phase, and total. (G) Quantitative real-time PCR showing relative mRNA level of Ucp1, Ucp2 and Ucp3 in the brown adipose tissue of animals on a HFD. (H) Core body temperature of animals on a HFD. Data are mean ± SEM. n=6–8/per group.* P<0.05 between control mice and liver PTG^{OE} mice fed a SD or HFD.
Figure 5. Effects of liver PTG overexpression on blood glucose, insulin levels, glucose tolerance, and insulin sensitivity. Control mice and liver PTG^OE^ mice aged 6 weeks were put on a SD or HFD for 16 weeks. (A) Blood glucose concentration in fed, 6-h fasted and 16-h fasted conditions. (B) Plasma insulin concentration in fed and 16-h fasted conditions. For glucose tolerance tests (GTT), mice were fasted for 16 h and injected with 2 g glucose/kg body weight, i.p. (C) GTT curve for glucose 12 weeks after being put on a SD or HFD, and glucose AUC. (D) GTT curve for insulin 12 weeks after being put on a SD or HFD, and insulin AUC. For insulin tolerance test (ITT), mice were fasted for 6 h and injected with 0.75 U insulin/kg i.p. (E) ITT 14 weeks after being put on a SD or HFD, expressed as absolute glucose. (F) ITT 14 weeks after being put on a SD or HFD, expressed as the percentage of initial values. Data are mean ± SEM. n=5–14/per group; * P<0.05 between control mice and PTG^OE^ mice fed a SD or HFD, ^ P<0.05 between control fed mice and control 16-h fasted mice fed a SD or HFD, # P<0.05 between a SD and HFD.

Figure 6. Liver PTG overexpression reduces HFD-induced hepatic steatosis. Control mice and liver PTG^OE^ mice aged 6 weeks were put on a SD or HFD for 16 weeks. Fed mice were sacrificed. (A) Fed liver triacylglycerol content. (B) Lipid deposition, as indicated by oil-Red-O staining in liver sections from fed mice in the SD and HFD group. (C) Quantitative real-time PCR showing relative mRNA level of SREBP1, GK, Fasn, Acc1α, and Pklr from the liver of mice fed a SD or HFD. (D) Quantitative real-time PCR showing relative mRNA level of PPARγ2 and MGAT1 in the liver of mice fed a SD or HFD. (E) Quantitative real-time PCR showing relative mRNA level of PPARα, Cpt1α, and Acox in the liver of mice fed a SD or HFD. Data are mean ± SEM. n=5–10/per group. .* P<0.05 between control mice and PTG^OE^ mice fed a SD or HFD. # P<0.05 between SD and HFD.
Figure 7. Fasting metabolic changes were attenuated in liver PTG-overexpressing animals. Control mice and liver PTG$^{OE}$ mice aged 6 weeks were put on a SD or HFD for 16 weeks. 16-h fasted mice were sacrificed. (A) Fasting serum non-esterified fatty acids. (B) Fasting serum β-hydroxybutyrate. (C) Fasting liver triacylglycerol content. (D) Lipid deposition, as indicated by oil-Red-O staining in liver sections from mice in the SD or HFD group. Data are mean ± SEM. n=5–8/per group; * P<0.05 between control mice and PTG$^{OE}$ mice fed a SD or a HFD. # P<0.05 between SD and HFD.
Diabetes

A) Body weight (g) over weeks.

B) Endothelial fat weight (g).

C) Subcutaneous fat weight (g).

D) Diameter (um).

E) Serum klin (ng/ml).

F) Food intake (Kcal/day).

G) Hypothalamic mRNA expression (arbitrary units).

H) Relative liver ATP content (fold of control).

279x361mm (300 x 300 DPI)
Diabetes

A

Liver triglyceride (mg/g liver)

Control   PTG<sup>OE</sup>

SD  HFD

B

Control SD  PTG<sup>OE</sup> SD

Control HFD  PTG<sup>OE</sup> HFD

C

mRNA levels (Relative units)

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E

mRNA levels (Relative units)

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