ORIGINAL

Resistance to high-fat diet-induced obesity in male heterozygous Pprc1 knockout mice

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Abstract. Peroxisome proliferator-activated receptor gamma, co-activator-related 1 (Pprc1) is the third member of the Pgc1 family. Other than the well-characterized Pgc1a and Pgc1b that act as regulators of mitochondrial biogenesis and oxidative metabolism, the function of Pprc1 *in vivo* is rarely reported, due to embryonic lethality of whole-body Pprc1 knockout mice. To investigate the biological and physiological function of Pprc1 in metabolic processes, male *Pprc1*^{+/-} mice fed with a high fat diet (HFD) showed resistance to diet-induced obesity with a decrease of adipose tissue in *Pprc1*^{+/-} mice, which was a result of elevated energy expenditure. In skeletal muscle of *Pprc1*^{+/-} mice, Pprc1 level showed haplo-insufficiency with down-regulation of Pgc1b and Pgc1a, whereas in adipose tissue, Pprc1 expression remained normal, with significant compensatory increase of other Pgc1 family members to induce an up-regulation of respiratory chain genes. Taken together, as the first report on the metabolic roles of Pprc1 *in vivo*, these results indicated an elevated basal metabolic rate and lipid metabolic alteration of male *Pprc1*^{+/-} mice on HFD, suggesting the significant role of Pprc1 in controlling mitochondrial gene expression and energy metabolic processes, synergistically with Pgc1a and Pgc1b.

Key words: Pprc1, Pgc1 family, High-fat diet, Obesity, Energy metabolism

WORLDWIDE prevalence of obesity and related metabolic diseases highlights the problem of energy balance and energy metabolism. Environmental factors, such as high-fat diets (HFDs), high caloric intake, and sedentary lifestyles, along with genetic variations, contribute to the pathogenesis of obesity. Pgc1a and Pgc1b potently induce the expression of a series of genes involved in energy homeostasis, and act as pleiotropic regulators of mitochondrial biogenesis and oxidative metabolism [1-4]. *Pgc1a*^{-/-} mice were protected from diet-induced obesity [5, 6], whereas transgenic mice of Pgc1a were severely obese [7]. In contrast, both transgenic and knockout of Pgc1b in mice were resistant to diet-induced obesity [8-10]. Meanwhile,

accumulating evidence associated genetic variations of these two members of Pgc1 family with human obesity and related diseases [2, 4, 11-16].

In vitro studies demonstrated that Pprc1, the third member of the Pgc1 family, interacts with transcription factors including Nrf1, Nrf2, Erra, Creb (cAMP-response element-binding protein), etc., and thus contributes to mitochondrial biogenesis and orchestrates response to metabolic stress by promoting the expression of multiple genes specifying inflammation, proliferation, and metabolic reprogramming [17-21]. Moreover, a human genetic analysis on type 2 diabetes showed a marginal significant association (OR = 1.06 [95% CI: 1.00-1.13], *P* = 0.06) between PPRC1 and type II diabetes, which suggested function of PPRC1 in human metabolic diseases [17]. Whole-body knockout of Pprc1 in mice causes embryonic lethality [18], hence reports on physiological function of the gene *in vivo* are quite rare. As the first report on metabolic roles of Pprc1 *in vivo*, the present study demonstrates that Pprc1^{+/-} mice are protected from HFD-induced obesity with a prominent decrease of adipose tissue as a

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result of elevated energy expenditure.

Materials and Methods

Mice

Pprc1^{+/-} mice were generated by homologous recombination as previously described [18]. Five-week-old male *Pprc1*^{+/+} and *Pprc1*^{+/-} littermate mice were randomized to either high-fat (HFD, D12492, Research Diets, New Brunswick, NJ, USA) or normal chow diet (ND) *ad libitum* for 13 weeks. Mice were housed individually for four consecutive days to compare food consumption and body temperature.

Experiments were performed with permission and followed the recommendations for the protection of Vertebrate Animals used for Experimental and Scientific purposes of the local Animal Care Committee in Shanghai Jiao-Tong University School of Medicine.

Metabolic tests of glucose homeostasis

Mice were fasted overnight prior to intra-peritoneal glucose tolerance test (IPGTT, 2 g glucose/kg body weight) and pyruvate tolerance test (PTT, 2 g sodium pyruvate/kg) or 8 hr before insulin tolerance test (ITT, 0.75 U insulin/kg). Blood glucose was monitored using OneTouch Ultra glucose meter (LifeScan, High Wycombe, Buckinghamshire, UK).

Body composition and indirect calorimetry

Nuclear magnetic resonance (NMR, Bruker Minispec, Hannover, Germany) was performed for body composition analysis. Oxygen consumption and carbon dioxide production were measured and correlated to individual body weights with Oxyman (Columbus Instruments International, Columbus, OH, USA), and then respiratory exchange ratio (RER) and heat were calculated accordingly [19].

Biochemical analysis

Enzymatic assay kits were used for determination of serum non-esterified fatty acid (NEFA, Wako, Osaka, Japan), triglyceride (Rsbio, Shanghai, China), cholesterol (Rsbio), and aspartate transaminase (AST, Rsbio) according to the manufacturer's instructions.

Real-time PCR

Total RNA was extracted from tissues of 22-week-old mice using Trizol Reagent (Invitrogen), and then reverse-transcribed with Reverse Transcription System

(Promega, Madison, WI, USA). Total DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen). Real-time PCR was performed with Sybr-Green (Takara, Shiga, Japan) on LightCycler 480 (Roche, Basel, Switzerland) using GAPDH for RNA and 18s RNA for mitochondrial DNA as internal reference. Primer sequences used are listed in Table S1.

Western blot

Brown adipocytes were lysed with 1% sodium deoxycholate, 1% Triton X-100 and protease inhibitor cocktail (Roche Applied Science). After 10 min of microcentrifugation at 20,000 x g in 4°C, the supernatant liquid was separated by SDS-PAGE, and then transferred to nitrocellulose membranes. Antigens were visualized by sequential treatment with specific antibodies. Goat anti-rabbit or anti-mouse IgG (1:4000; ZSGB-BIO Inc., Beijing, China) were used as the secondary antibody. Immunohistochemistry immune complexes were detected by using Immobilon Western Chemiluminescent HRP Substrate kit (Merck Millipore, Darmstadt, Germany).

Statistical analysis

Data are expressed as mean ± SEM. Statistical analyses were performed with Student's *t* test for independent samples. For growth curve analysis, one-way repeated-measures ANOVA and Bonferroni multiple comparisons test were performed. **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

Results

Growth curves and body composition in *Pprc1*^{+/-} mice

Given the fact that homozygous mutation of *Pprc1* causes embryonic lethality, *Pprc1*^{+/-} mice were generated from homologous recombination as previously reported [18]. When fed on ND, *Pprc1*^{+/-} mice appeared normal, with body weights similar to wild-type (WT) littermates (Fig. 1a). However, *Pprc1*^{+/-} mice showed significantly less weight gain than WT on HFD (Fig. 1b), which was consistent with fat content reduction (-15.3%, *p* = 0.0244) of *Pprc1*^{+/-} mice (Fig. 1c). Meanwhile, the relative lean mass of *Pprc1*^{+/-} mice increased significantly (+19.0%, *p* = 0.0080 in male; +39.4%,) compared to WT (Fig. 1d).

Glucose homeostasis and serum chemistry in *Pprc1*^{+/-} mice

Body mass reduction may occur as a result of glu-

cose homeostasis disorder. IPGTT, ITT and PTT were conducted in *Pprc1*^{+/-} and WT mice on both ND and HFD, but showed no differences (Fig. S1a-c). Serum chemistry profiles further revealed that *Pprc1*^{+/-} mice had almost identical T-C, HDL-C, LDL-C, NEFA, and AST levels with WT, and yet TG level showed a 105.7% increase ($p = 0.0001$) (Fig. S1d-g).

Elevated energy consumption in *Pprc1*^{+/-} mice on HFD

Unbalanced energy intake and expenditure contribute to obesity and leanness. However, *Pprc1*^{+/-} mice had comparable food consumption (Fig. 1k) and body temperature (Fig. 1j) with WT on HFD. Subsequently, energy expenditure of the mice was studied with indirect calorimetry. Increased O₂ consumption (Fig. 1e) and CO₂ production (Fig. 1f) both in daylight and in dark and higher heat consumption (Fig. 1i) were evident, indicating significantly increased energy expenditure in *Pprc1*^{+/-} mice on HFD. In addition, respiratory exchange ratio (RER) of *Pprc1*^{+/-} mice decreased (from 0.789 to 0.782, $p = 0.0062$) in light cycle (Fig. 1i-j), with unaltered locomotor activity (Fig. 1h), suggesting an increase of fat oxidation in *Pprc1*^{+/-} mice on HFD.

Decreased adipose tissue and mitochondrial content alternation in *Pprc1*^{+/-} mice on HFD

Dissection of fat deposits further showed that *Pprc1*^{+/-} mice were protected from diet-induced obesity. Subcutaneous white adipose tissue (WAT) (sub-WAT; -55.6%, $p = 0.0349$) (Fig. 2a) were significantly decreased in *Pprc1*^{+/-} mice. Interestingly, brown adipose tissue (BAT) was also decreased (-22.2%, $p = 0.0068$) (Fig. 2b). Histology HE staining showed decreased lipid droplet diameter of *Pprc1*^{+/-} mice both in BAT and subWAT (compared with their littermates (Fig. 2c). In BAT, expression analysis of mitochondrial content showed a 3.72-fold ($p=0.0013$) increase in *Pprc1*^{+/-} mice (Fig. 2o), consisted with the increased protein level of UCP1 (Fig. 2h.i). It suggested that BAT is a vital organ for the leanness phenotype of these mice.

Altered expression profiles in *Pprc1*^{+/-} mice on HFD

Expression analysis of Pgc1 family genes (Fig. 2e-g) revealed that in *Pprc1* highly expressed tissues [20], *Pprc1*^{+/-} mice on HFD did not necessarily show an insufficient expression. Meanwhile, the compensatory increase of Pgc1b (1.53-fold, $p = 0.0256$) in WAT (Fig. 2e) and Pgc1a (4.98-fold, $p = 0.0254$) in BAT (Fig. 2f) was consistent with previously reported up-regulation

of Pgc1a in the absence of Pgc1b [17, 21]. In skeletal muscle, the expression of *Pprc1* decreased by 66.2% ($p = 0.0402$), along with a 57.9% decrease ($p = 0.0256$) of Pgc1b expression (Fig. 2g). Furthermore, energy metabolism-related gene expression was detected. Although there was no significant difference of analyzed genes between *Pprc1*^{+/-} and WT mice in skeletal muscle (Fig. 2m), expression levels of myosin heavy chain (MHC) isoforms showed a down-regulation of MHC1 (74.6%, $p<0.05$) and a 5-fold up-regulation of MHC IIb ($p<0.05$) (Fig. 2o), which indicated a less oxidative and more glycolytic alternation in soleus muscle in *Pprc1*^{+/-} mice. In BAT of *Pprc1*^{+/-} mice (Fig. 2i), the compensatory increase of Pgc1a expression was followed by significant up-regulation of Cox2, Cox5b, Gpam and PPAR γ 2. In WAT the up-regulation of leptin and Ucp1 followed the increased expression of Pgc1b. The up-regulation of respiratory chain mRNA and lipid metabolism-related mRNA suggested increased energy expenditure in these tissues.

Decreased liver lipid with expression profiles alternation in *Pprc1*^{+/-} mice on HFD

Dissection showed unaltered liver weight in *Pprc1*^{+/-} mice compared with their littermates on HFD (Fig. 3a), whereas histology HE staining showed decreased lipid droplet diameter in *Pprc1*^{+/-} mice (Fig. 2d). Though no difference was observed in expression analysis of Pgc1 family genes (Fig. 2b), leptin, Gpam, and COX2b showed increased levels in *Pprc1*^{+/-} mice (Fig. 2c). It suggested an up-regulation of both lipid biogenesis and consumption in *Pprc1*^{+/-} mice on HFD, which might be an adaptation to the elevation of serum TG (Fig. S1e) and the reduction of hepatic TG (Fig. 3e).

Discussion

The PGC1 family members are dynamically regulated in response to various signaling pathways involved in nutrient sensing and energy balance. PGC1a plays a key role as regulator of adaptive thermogenesis through UCP1 [22], and interacts with CREB to regulate gluconeogenesis [23]. Meanwhile, PGC1b co-activates SREBPs to stimulate lipogenic gene expression [1], and contributes to insulin sensitivity in liver [20, 24]. Regulated by AMPK and SIRT1, PGC1a bridges mitochondrial biogenesis to metabolic signaling pathways [25]. Targeted disruption of either Pgc1a [5, 6, 26] or Pgc1b [8, 9] in mice protects from obesity on both ND and HFD. Due to the fetal lethality of *Pprc1*^{-/-} mice

Fig. 1a

Fig. 1b

Fig. 1c

Fig. 1d

Fig. 1e-1

Fig. 1e-2

Fig. 1f-1

Fig. 1f-2

Fig. 1g-1

Fig. 1g-2

Fig. 1h-1

Fig. 1h-2

Fig. 1i-1

Fig. 1i-2

Fig. 1j

Fig. 1k

Fig. 1 Growth curves of male *Pprc1*^{+/-} (*Pprc1*-HET) mice and wild-type littermates (WT) on ND (a) ($n = 13-20$) and HFD (b) ($n = 12-17$) are shown. Fat (c) and lean (d) mass measured by NMR in 20-week-old mice both on ND and HFD ($n = 8$) O_2 consumption (e), CO_2 production (f), RER (g), and heat (i) of *Pprc1*^{+/-} mice and wild-type littermates on HFD at 20 weeks old were recorded and calculated during the final 24 h of the procedure. Body temperature (j) ($n = 8$) and food consumption (k) ($n = 4-8$) of mice on HFD were calculated individually for four consecutive days. Bar charts represent the average of each group ($n = 8$). Error bars show S.E.M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; WT vs. *Pprc1*-HET mice; Student's t test (c-k) or one-way repeated-measures ANOVA (a-b).

Fig. 2a

Fig. 2b

Fig. 2c

Fig. 2d

Fig. 2e

Fig. 2f

Fig. 2g

Fig. 2h

Fig. 2i

Fig. 2k

Fig. 2j

Fig. 2m

Fig. 2n

Fig. 2o

Fig. 2 Comparison of tissue weights of subcutaneous white adipose tissue WAT in lower limb segments (subWAT) (a), interscapular brown adipose tissue (BAT) (b) between 22-week-old male *Pprc1*^{+/-} mice and wild-type littermates on HFD (n = 8-9). Representative histological images of subWAT and BAT stained with HE in *Pprc1*^{+/-} mice and wild-type littermates (WT) on HFD (c), Scale bars, 50 μ m. Average lipid-droplet diameter was measured in WAT (d) (n=4-5). Relative mRNA levels of Pgc1 family genes in WAT (e), BAT (f), and soleus muscle (g). Relative mRNA levels of energy metabolism related gene in BAT (j), WAT (k) and soleus muscle (m), relative abundance of mitochondrial DNA in BAT, WAT, and soleus muscle (n). Individual measurements were standardized using GADPH (j-m) or D-loop (n), and then the average of the WT group was set to 1 (n = 5-8). Relative mRNA levels of myosin heavy chain (MHC) isoforms (o) in soleus muscle, adjusted GADPH, and then the average of the WT group was set to 1 (n=4-5). Western blotting of UCP1 protein levels in BAT (h) in *Pprc1*^{+/-} mice and wild-type littermates (WT) on HFD (n=4). The right panel represents relative quantification of UCP1 protein (i), normalized to β -tubulin, and then the average of the WT group was set to 1. Error bars show S.E.M. *p < 0.05, **p < 0.01; WT vs. *Pprc1*-HET mice; Student's *t* test.

Fig. 3 Comparison of tissue weights of liver (a) between 22-week-old male *Pprc1*^{+/-} mice and WT littermates on HFD (n = 8-9). Relative mRNA levels of *Pgc1* family genes (b) and energy metabolism related gene (c) in liver (b). Individual measurements were standardized using *GADPH*, and then the average of the WT group was set to 1 (n = 5-8). Representative histological images of liver stained with HE in *Pprc1*^{+/-} mice and WT littermates on HFD (d), Scale bars, 50 μ m. Hepatic triglyceride (d) on HFD (n = 8-9). Error bars show S.E.M. **p* < 0.05, ***p* < 0.01; WT vs. *Pprc1*-HET mice; Student's *t* test.

[18], the present study is able to investigate the metabolic alteration of *Pprc1*^{+/-} mice, and find that *Pprc1*^{+/-} mice are resistant to diet-induced obesity, which is the result of an elevated energy expenditure.

Different from those on ND, male *Pprc1*^{+/-} mice on HFD display decrease of body weight and fat mass, along with increase of relative lean mass and heat consumption (Fig. 1). With unaltered locomotor activity, adipocyte tissue was treated as the main organ for the leaner phenotype of *Pprc1*^{+/-} mice. Decrease in relative weights of fat deposits and alternation of adipocyte lipid droplets in both WAT and BAT confirm the remarkable alterations occurring in adipose tissue (Fig. 2). Although the biological and physiological functions of *Pprc1* remain largely unknown, there is evidence to suggest the potential role of this gene in metabolic processes. Induced by serum stimulation, *Pprc1* is an immediate early gene that is rapidly induced in proliferative cell growth [27, 28]. *Pprc1* expression consistently peaks during the first day of embryoid body formation, which suggests that *Pprc1* may support the high rate of mitochondrial transcription taking place during early embryogenesis [18]. A microarray screening further revealed that *Pprc1* responds to multiple metabolic insults and induces a series of genes involved in inflam-

mation, cell stress, and proliferation [29].

It is reported that *Pgc1* α and *Pgc1* β coordinates the formation of slow-twitch muscle fibers [30, 31], whereas only in skeletal muscle (soleus) of *Pprc1*^{+/-} mice, *Pprc1* mRNA level shows haplo-insufficiency, with down-regulation of *Pgc1* α and *Pgc1* β , other than in WAT and BAT (Fig. 2). Energy needs in muscle are quite high as living organisms convert chemical energy into mechanical work with the help of muscle [4]. As different muscle fiber types (Type I, Type IIa, Type IIb, and Type IIx) can be distinguished by the type of myosin heavy chain (MHC) isoforms [32-34], increased MHC2b and decreased MHC1 in *Pprc1*^{+/-} suggests an alternation of fiber type from "slow twitch oxidative" to a more "fast twitch glycolytic" [35]. As high level expression of MHC1 in skeletal muscle is related with low-energy expenditure, the decrease of MHC1 and increase of MHC2b (Fig. 2o) in skeletal muscle comprise a high energy consumption in *Pprc1*^{+/-} mice. Though mitochondrial volume remains unaltered, some expression of genes of oxidative phosphorylation increased. Consistent with the phenotype of *PGC1* α -ko mice [36], it suggests that *Pprc1*, as well as other *Pgc1*s, could participate in the energy consumption process in a tissue-specific manner. On the other hand, consistent with a previ-

ously reported up-regulation of Pgc1a in the absence of Pgc1b [8, 9], our results further show the compensatory increase of other Pgc1 family member that could induce an up-regulation of respiratory chain mRNA in *Pprc1*^{+/-} mice, suggesting a distinctively co-operative and compensatory relationship prevailing among the three Pgc1 family members in physiological condition.

Interestingly, this study provides yet another example of increasing serum TG level in leaner mice, in addition to the one previously reported [37]. The increase of serum TG in *Pprc1*^{+/-} mice is consistent with the up-regulation of Gpam expression in BAT and in liver (Fig. 2i, 3c). Gpam is supposed to initiate hepatic *de novo* TG synthesis both *in vitro* [38, 39, 26] and *in vivo* [40]. Liver-directed overexpression of Gpam in mice results in increased plasma TAG [41]. However, histological examination of liver tissue from *Pprc1*^{+/-} mice revealed a reduction in lipid droplets compared with their littermates (Fig. 3d). Consistent with this histology, a tendency of reduction in hepatic triglycerides was seen (Fig. 3e), as well as an increase in hepatic expression of fatty acid oxidation genes (Fig. 3c). This suggests the possibility that up-regulated hepatic fatty acid oxidation and increased hepatic *de novo* TG syn-

thesis happened simultaneously in *Pprc1*^{+/-} mice on HFD, and that oxidation may have exceeded synthesis.

Taken together, these observations demonstrate an elevated basal metabolic rate and lipid metabolic alteration of *Pprc1*^{+/-} mice on HFD, and indicate a significant role of Pprc1 in controlling mitochondrial gene expression and energy metabolic process, synergistically with Pgc1a and Pgc1b.

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

Supplementary Table 1 Sequences for real-time PCR primers

Target	Forward primer (5'→3')	Reverse primer (5'→3')
Gapdh	TGACCACAGTCCATGCCATC	GACGGACACATTGGGGGTAG
Pgc1α	TATGGAGTGACATAGAGTGTGCT	CCACTTCAATCCACCCAGAAAG
Pgc1β	TCCTGTAAAAGCCCGGAGTAT	GCTCTGGTAGGGGCAGTGA
Pprc1	CAGGAGAAGAAGCCCTTAGACC	CTTTCGCCAAGAGTGAGACAG
Cox2	GCTCCTTAGTCTCTATATCAT	TGCGTCTATTGTGCTTGTA
Cox4i	CCATGTCACGATGCTGTCTG	GATGCGGTACAACCTGAACCTTCT
Cox5b	GGAAGACCCTAATCTAGTCCCG	GTTGGGGCATCGCTGACTC
Tfam	ATCCGAAGTGTTCCTCAGCA	TCTGAAAGTTTGCATCTGGGT
Cytb	GGACGAGGCTTATATTATGGA	AATGCTGTGGCTATGACTG
Glut4	GTGACTGGAACACTGGTCCTA	CCAGCCACGTTGCATTGTAG
Ucp1	AGGCTTCCAGTACCATTAGGT	CTGAGTGAGGCAAAGCTGATTT
Ucp3	CTGCACCGCCAGATGAGTTT	ATCATGGCTTGAAATCGGACC
Cpt1b	AGGCACTTCTCAGCATGGTC	GCTTCAGGGTTTGTCGGAAGA
Dgat1	GTGCCATCGTCTGCAAGATTC	GCATCACCACACACCAATTCAG
Fas	GCGGGTTCGTGAACTGATAA	GCAAAATGGGCCTCCTTGATA
Gpam	TCTCTGGGTTTGCGGAATGTT	GCCTTGTGTGCGTTTCATTGAT
Scd1	TCTTGCGATACACTCTGGTGC	CGGATTGAATGTTCTTGTCTGCT
PPARγ1	GTCACACTCTGACAGGAGCC	AGAACGTGACTTCTCAGCCC
PPARγ2	TCTCCTGTGACCCAGAGCAT	CCACAGAGCTGATTCCGAAGT
Creb1	AGCAGCTCATGCAACATCATC	AGTCCTTACAGGAAGACTGAACT
Fabp4	AAGGTGAAGAGCATCATAACCCCT	TCACGCCTTTCATAACACATTCC
Leptin	GTTAGACCCTACTCACGGCG	TGTGCCCTGAAATGCGGTAT
MHC I	CCAAGGGCCTGAATGAGGAG	GCA AAG GCT CCA GGT CTG AG
MHC II a	AAG CGA AGA GTA AGG CTG TC	GTG ATT GCT TGC AAA GGA AC
MHC II b	CGA AGG CGG AGC TAC GGT CA	CGG CAG CCA CTT GTA GGG GT
MHC II x	GCC AGG GTC CGT GAA CTT GAA G	CCT CCG CTT CCT CAG CTT GTC T
18S	ACCGCAGCTAGGAATAATGGA	ACCTAGTTCCGAAAACCA

Supplementary Fig. S1 Intra-peritoneal glucose tolerance test (IPGTT) (a), intra-peritoneal insulin tolerance test (ITT) (b) and intra-peritoneal pyruvate tolerance test (PTT) (c) of *Pprc1*^{+/-} mice and wild-type littermates on ND and HFD at 21 weeks old. The bar charts represent the average area under the curve (AUC) (n = 7-8). Serum cholesterol (d), triglyceride (TG) (e), non-esterified fatty acid (NEFA) (f), and aspartate transaminase (AST) (g) of 22-week-old male *Pprc1*^{+/-} mice and wild-type littermates on HFD (n = 8-9). Error bars show S.E.M. ***p* < 0.01; WT vs. *Pprc1*-HET mice.

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