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—Original—

Porcine Adiponectin Receptor 1 Transgene Resists High-fat/Sucrose Diet-Induced Weight Gain, Hepatosteatosis and Insulin Resistance in Mice

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Abstract: Adiponectin and its receptors have been demonstrated to play important roles in regulating glucose and lipid metabolism in mice. Obesity, type II diabetes and cardiovascular disease are highly correlated with down-regulated adiponectin signaling. In this study, we generated mice overexpressing the porcine *Adipor1* transgene (*pAdipor1*) to study its beneficial effects in metabolic syndromes as expressed in diet-induced obesity, hepatosteatosis and insulin resistance. Wild-type (WT) and *pAdipor1* transgenic mice were fed ad libitum with a standard chow diet (Chow) or a high-fat/sucrose diet (HFSD) for 24 weeks, beginning at 6 to 7 weeks of age. There were 12 mice per genetic/diet/sex group. When challenged with HFSD to induce obesity, the *pAdipor1* transgenic mice resisted development of weight gain, hepatosteatosis and insulin resistance. These mice had lowered plasma adiponectin, triglyceride and glycerol concentrations compared to WT mice. Moreover, we found that (indicated by mRNA levels) fatty acid oxidation was enhanced in skeletal muscle and adipose tissue, and liver lipogenesis was inhibited. The *pAdipor1* transgene also restored HFSD-reduced phosphoenolpyruvate carboxykinase 1 (*Pck1*) and glucose transporter 4 mRNA in the adipose tissues, implying that the increased *Pck1* may promote glyceroneogenesis to reduce glucose intolerance and thus activate the flux of glyceride-glycerol to resist diet-induced weight gain in the adipose tissues. Taken together, we demonstrated that *pAdipor1* can prevent diet-induced weight gain and insulin resistance. Our findings may provide potential therapeutic strategies for treating metabolic syndromes and obesity, such as treatment with an ADIPOR1 agonist or activation of *Adipor1* downstream targets.
Key words: adiponectin receptor 1, diet-induced obesity, insulin resistance, pig

Introduction

Chronic intake of a high-fat/sucrose diet (HFSD) is one environmental factor responsible for the development of metabolic syndromes, including type II diabetes, insulin resistance, atherosclerosis and inflammation [1, 33, 35]. In both obese and lean mice, HFSD was induces glucose intolerance and correlates with plasma concentrations of adipokines [36]. Adipose tissues secrete a variety of factors or adipokines, such as leptin, adiponectin, resistin, interleukin-6 and tumor necrosis factor a, which have been demonstrated to play important

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roles in regulating insulin resistance and metabolic homeostasis. Abdominal obesity is highly correlated with plasma concentrations of these adipokines in human metabolic disorders [11, 16, 42].

Adiponectin is an anti-inflammatory adipokine that increases fatty acid oxidation, decreases gluconeogenesis, improves insulin sensitivity and regulates food intake [20]. Secretory adiponectin, especially the high-molecular-weight form, is abundant in the circulation, and is negatively associated with obesity and type II diabetes mellitus [41]. AMP-activated protein kinase (AMPK) is downstream effector mediating adiponectin action through two major receptors, adiponectin receptors 1 (ADIPOR1) and 2 (ADIPOR2) [41]. In a gene knock-out study *Adipor2*$^{-/-}$ mice are lean and resistant to a high-fat diet-induced obesity and glucose intolerance, whereas *Adipor1*$^{-/-}$ may have the opposite functions [6].

Although adenovirus infection has been used to study the functions of ADIPOR1 and ADIPOR2 [43], the molecular mechanism underlying these two receptors in diet-induced metabolic syndrome remains unclear. This laboratory has been interested in porcine adipose tissue lipid metabolism and its regulation, including studies of the cloned *pAdipor1* and *pAdipor2*. Our previous study found that both ADIPOR1 and ADIPOR2 were highly homologous between pigs and mice, and the receptors responded to insulin via the phosphatidylinositol 3-kinase (PI3K) pathway [10, 18, 19]. We have expressed the pADIPOR1 in mice in order to ascertain its metabolic functions and to be able to compare its functions to mADIPOR1. The association of the pADIPOR1 and energy utilization in differ tissues have not been demonstrated. We proposed that pADIPOR1 may act as mADIPOR1 to mediate adiponectin's function. Therefore, in the current study, the *pAdipor1* transgenic mice were challenged with a HFSD to study underlying mechanisms in diet-induced metabolic syndromes.

Materials and Methods

Generation of pAdipor1 transgenic mice

The cDNAs of *pAdipor1*(Genbank no. AY578142) containing a N-terminal Kozak sequence and a C-terminal FLAG-tag were constructed into the Vitality® pIRES-hrGFP II Mammalian Expression Vector (Stratagene, La Jolla, CA, USA) by swapping the CMV promoter with that of the chicken β-actin (pCX-EGFP). The *pAdipor1* is widely expressed and most abundant in the heart and skeletal muscle [10]. Hence, we utilized the chicken β-actin promoter to constantly drive the expression of *pAdipor1* in mice. The humanized recombinant green fluorescent protein region was replaced with the red fluorescent protein coding region (pTRE-Tight-DsRed2 expression vector; BD Biosciences Clontech, San Jose, CA, USA) for the construction of the *pAdipor1* transgene (also see Supplemental Fig. 1: refer to J-STAGE at https://www.jstage.jst.go.jp/browse/expanim). The *DsRed2* was used as a reporter gene for genotyping the heterozygous and homozygous transgenic mice at birth and has no obvious negative effects *in vivo* [21, 32, 39].

Both C57BL/6J and FVB/N mice are commonly used inbred mouse strains and are susceptible to diet-induced obesity [25]. Here, the fertilized eggs from FVB/N donors were used because of better reproductive performance than C57BL/6J mice. The injection fragment (the β-actin-driven *pAdipor1/DsRed2*) was digested with HinCII and PsiI to remove the neomycin/kanamycin resistance genes, separated by agarose gel-electrophoresis, purified, and used for pronuclear microinjection. The founder mice were crossed with WT to generate the F_1 heterozygous (*pAdipor1*$^{+/-}$) offspring and then backcrossed to the WT to generate the F_2. When the F_2 progeny were crossed, quantitative real-time PCR (qPCR) and Southern blotting analyses were performed to identify the homologous (*pAdipor1*$^{+/+}$) littermates of the F_3 [34]. For qPCR analysis, primer pairs of DsRed2 (Table 1) were used to avoid interference by intrinsic gene expression in *pAdipor1* transgenic mice.

Ultimately, we obtained two *pAdipor1/DsRed2* lines of transgenic mice with different insertion sites, but a similar expression pattern by ANOVA analysis (expression of membrane transgenes confirmed by the ADIPOR1 polyclonal antibody targeting both mouse and pig gene products, Supplemental Fig. 1). All experiments were carried out on both male and female mice with homologous offspring from the F_4 or later generations (n=6 for each line/sex).

Induction of obesity

Mice of 6 to 7 weeks old were randomly housed in cages (n=6 per cage) for each experimental group (n=12, two lines) with the light-dark cycle maintained at 12:12 h (lighting from 06:00 to 18:00 h). The wild-type (WT) mice, two lines of *pAdipor1* transgenic mice were fed

Table 1. Primer sets for qPCR

Gene name	Primers 5'-3'(forward and reverse)	Length (bp)	Annealing temperature (°C)	Reference sequence
DsRed2	F: GACCCACAAGGCCCTGAAG, R: TGCTCCACGATGGTGTAGTCC	159	64	EU016077
Adiponectin	F: GGCTCTGTGCTGCTCCATCT, R: AGAGTCGTTGACGTTATCTGCATAG	101	55	NM_009605
Adipor1	F: CCTGGCTCTATTACTCCTTC, R: GAACACTCCTGCTCTTGTCT	149	62	NM_028320
Pparα	F: TGCTGGTATCGGCTCAATAA, R: TCCTGCCACTTGCTCACTAC	114	64	NM_011144
Acox1	F: AGTTCCAACTAGCCAGGCAT, R: GAGTGGCCTTGACCTCTGAT	81	62	NM_015729
Cpt1a	F: GGTCTCAAGTAATGGGTGC, R: GAATACCAAACGGAGTTGC	102	62	BC054791
Cpt1b	F: TTTGGGAACCACATCCGCCAA, R: TTATGCCTGTGAGCTGGCCAC	262	60	NM_009948
CD36	F: CAAGCTCCTTGGCATGGTAGA, R: TGGATTTGCAAGCACAATATGAA	92	62	NM_007643
Ucp2	F: CTCTTCTCTGGGAGCCAATC, R: CCCCTTCACCTCTTTAGCAG	99	62	NM_011671
Srebf1	F: GAACCAGCGGTGGGAACACAGAGC, R: GACGGCGGCAGCTCGGGTTTCTC	224	57	NM_011480
Fasn	F: GGGCACTGACTGTCTGTTTTCC, R: GGATCAGGAGAGCATCAAGAGC	200	60	NM_007988
Glut4	F: TACATACCTGACAGGGCAAGG, R: TTCGGGTTTAGCACCCTTC	131	58	NM_009204
Pck1	F: GTCACCATCACTTCCTGGAAGA, R: GGTGCAGAATCGCGAGTTG	174	64	NM_011044
β-actin	F: TGTTACCAACTGGGACGACA, R: CTTTTCACGGTTGGCCTTAG	130	62	NM_007393

ad libitum with either a standard chow diet (Chow) containing 3.5 kcal/g metabolic energy (MF-18: 18% protein, 18% fat, 6% fiber, and 58% nitrogen free extract; Oriental Yeast Co., Tokyo, Japan) or a HFSD consisting (on a weight basis) of 21.3% protein, 23.6% fat, 5.8% fiber, and 41.2% carbohydrates with 4.65 kcal/g metabolic energy (45% energy from fat; St. Louis, MO, USA). Body weights of each feeding group were measured every two weeks.

Sample collection

After 25 weeks of feeding, the mice were anesthetized with 2,2,2-tribromoenthanol (intraperitoneally) and blood samples from tail vein were collected with EDTA anticoagulant for determining plasma adiponectin, insulin, triglycerides and glycerol levels. Animals were then sacrificed by CO_2 and the perigonadal adipose tissues (epididymal in males and ovarian/uterine in females), skeletal muscles and livers were excised, frozen in liquid nitrogen and stored at −80°C until RNA extraction and histological examination. Plasma and tissue samples were collected from mice at 09:00~12:00 after a 12 h fast. The animal protocol was approved by the Experimental Animal Care and Use Committee at National Taiwan University.

Intraperitoneal glucose tolerance test (IPGTT)

After 24 weeks, an IPGTT was performed at 10:00 h after a 12 h fasting by injecting mice (n=6 per group) intraperitoneally with 2 mg/g body weight of glucose. Blood samples were taken from the tail vein at 0, 15, 30, 60 and 120 min after glucose injection for the determination of plasma glucose levels (Accu-Chek® Active; Roche Diagnostics, Mannheim, Germany).

Plasma adiponectin, insulin, triglyceride and glycerol levels

Plasma adiponectin level was determined using a

Mouse/Rat Adiponectin ELISA Kit (UM-100201, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). Plasma insulin was measured by a mouse insulin ELISA kit (10-1149-01, Mercodia, Uppsala, Sweden). Plasma triglyceride was measured by a Triglyceride Colorimetric Assay Kit (10010303, Cayman Chemical Co., Ann Arbor, MI, USA). Plasma glycerol was measured by a Glycerol Colorimetric Assay Kit (10010755, Cayman Chemical Co. Ann Arbor, MI, USA). All plasma samples were assayed in duplicate and determinations were according to the manufacturer's instructions.

Liver histology

Liver frozen for histology was imbedded in Optimum Cutting Temperature Compound (4583, Sakura Finetek USA, Inc., Torrance, CA, USA) and sectioned at 6-μm thickness. The liver tissue slices were then fixed in 10% (v/v) buffered formalin and stained with hematoxylin and Oil Red O for the detection of lipid droplets [31]. The lipid contents were then quantified using ImageJ 1.46r software.

Quantitative PCR (qPCR) analysis

Total RNAs were extracted from tissue samples in TRI REAGENT® (Molecular Research Center, Inc., Cincinnati, OH, USA) by homogenization using a ZrSiO beads-based homogenizer (Next Advance Inc., Averill Park, NY, USA). For the qPCR analysis, first-strand cDNA was synthesized from TURBO™ DNase-treated (Applied Biosystems, Foster, CA, USA) total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The cDNA for individual genes was amplified using the RealQ-PCR Master Mix Kit (250507, Ampliqon, Copenhagen, Denmark) with paired forward and reverse primers (Table 1) designed from UniSTS database in National Center for Biotechnology Information. Amplification of specific transcripts was further confirmed by melting curve profile analysis. The *pAdipor1* primers could detect both mouse and pig target genes. The relative expression levels were calculated according to the formula $2^{-\Delta CT}$ and normalized using the expression of the β-actin housekeeping gene in the same sample.

Statistical analysis

Numerical values were expressed as the mean ± SEM. Results involving more than two groups (genotype, diet, time and sex effects) were assessed by two-way ANOVA procedure. The Dunnett's post-hoc test was followed to evaluate differences among means (SAS Inst., Inc., Cary, NC, USA) for multiple comparisons. A significant difference was indicated at $P \leq 0.05$.

Results

pAdipor1 transgenic mice were small and resistant to HFSD-induced hepatosteatosis

The *pAdipor1* transgenic mice were smaller than WT mice (Fig. 1A and B) and deposition of white adipose tissue around with gonads was limited (data not indicated). In Chow-fed mice, total adipose tissue *Adipor1* and *Adipor2* mRNA levels were much greater than in other tissues (Supplemental Fig. 1B). Total *Adipor2* mRNA in liver and muscle were depressed. Consistent with its role in modulating energy homeostasis, we found that the *pAdipor1* transgenic mice fed the Chow diet were significantly smaller than the WT mice (Fig. 1A and B). HFSD induced obesity in the WT mice (with a stronger effect in the males); the *pAdipor1* transgenic mice were leaner than the WT mice (Fig. 1A and B). More importantly, while HFSD yielded extensive hepatic fat deposition in the WT mice (55% increase in lipid content − data not indicated), this symptom was not observed in *pAdipor1* transgenic mice (Fig. 1C; male), confirming the beneficial effects of *pAdipor1* transgene on hepatosteatosis.

Glucose intolerance is not seem in HFSD-fed pAdipor1 transgenic mice

As shown in Fig. 2B, 24-week-HFSD-feeding induced hyperglycemia in WT mice. However, only the male mice developed higher fasting insulin levels, indicating that insulin resistance was developing in this treatment group (Fig. 2A). By sharp contrast, HFSD-fed *pAdipor1* transgenic mice had lower fasting plasma glucose levels than HFSD-fed WT mice (Fig. 2B). Furthermore, the highly elevated plasma insulin levels seem in male HFSD-fed WT mice were not present in *pAdipor1* transgenic mice, confirming an anti-diabetic role for *pAdipor1* transgene (Fig. 2A and 2B).

Plasma triglyceride levels (Fig. 2C) were lower in *pAdipor1* transgenic male mice than in WT male mice when fed Chow. HFSD lowered plasma triglyceride in male WT mice and in female *pAdipor1* transgenic mice. These results suggest that the male mice were more sensitive to the HFSD challenge and had poorer glucose

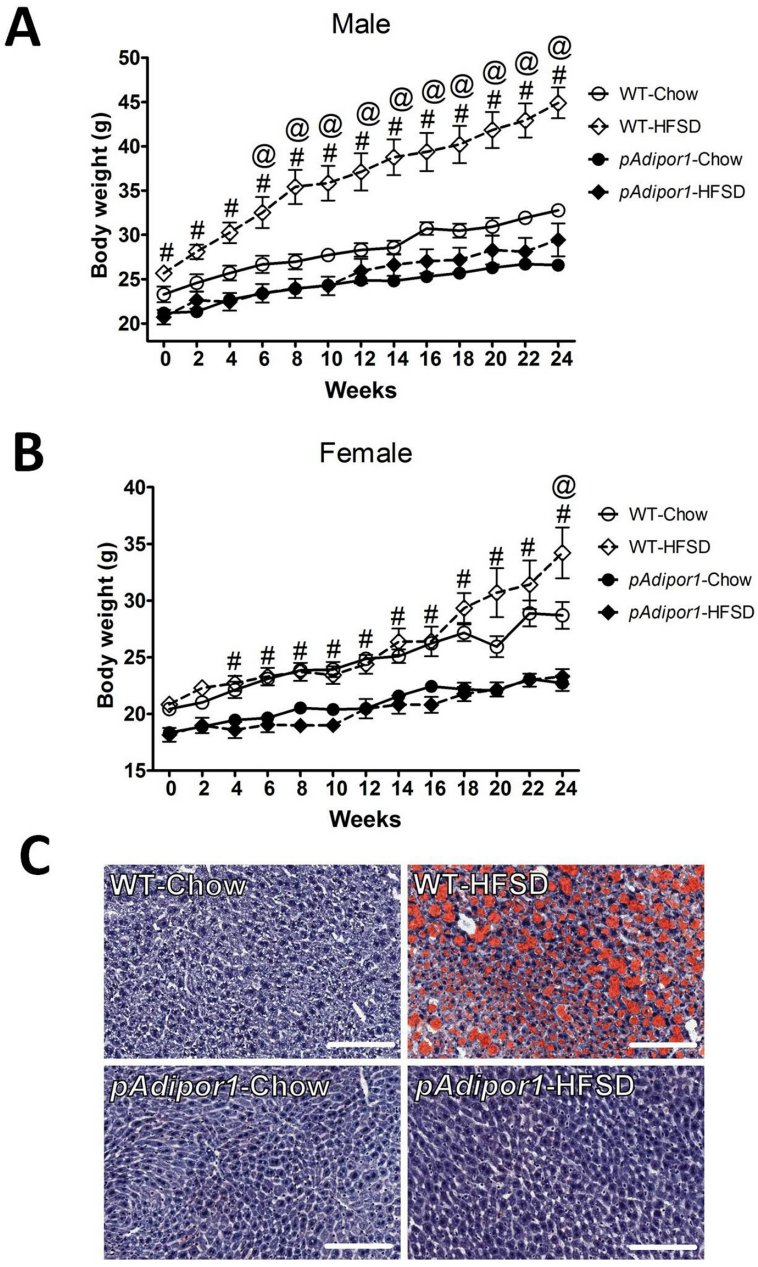

Fig. 1. The effects of *pAdipor1* transgene on HFSD-induced obesity. Wild-type (WT) and *pAdipor1* transgenic mice were fed *ad libitum* a standard chow diet (Chow) or a high-fat/sucrose diet (HFSD) for 24 weeks, beginning at 6–7 weeks of age. There were 12 mice per genetic/diet/sex group. A: Body weights for the male mice. B: Body weights for the female mice. C: Histological sections of the livers (male) stained with Oil Red O to detect hepatic lipid droplets. Values were expressed as mean ±SEM. Differences among means for multiple comparisons were evaluated by two-way ANOVA and post-hoc test. $P \leq 0.05$: #, WT-HFSD vs. *pAdipor1*-HFSD; @, WT-HFSD vs. WT-Chow. The scar bars represented 100 μm.

tolerance and thus a greater tendency to develop obesity and diabetes than the females. We also found that for the males, the plasma levels of total adiponectin were down-regulated in *pAdipor1* transgenic mice, regardless of the dietary treatment (Fig. 2D).

We further quantified the degree of glucose intolerance using IPGTT and found that HFSD fed male WT mice showed impairment in glucose tolerance (Fig. 2E and

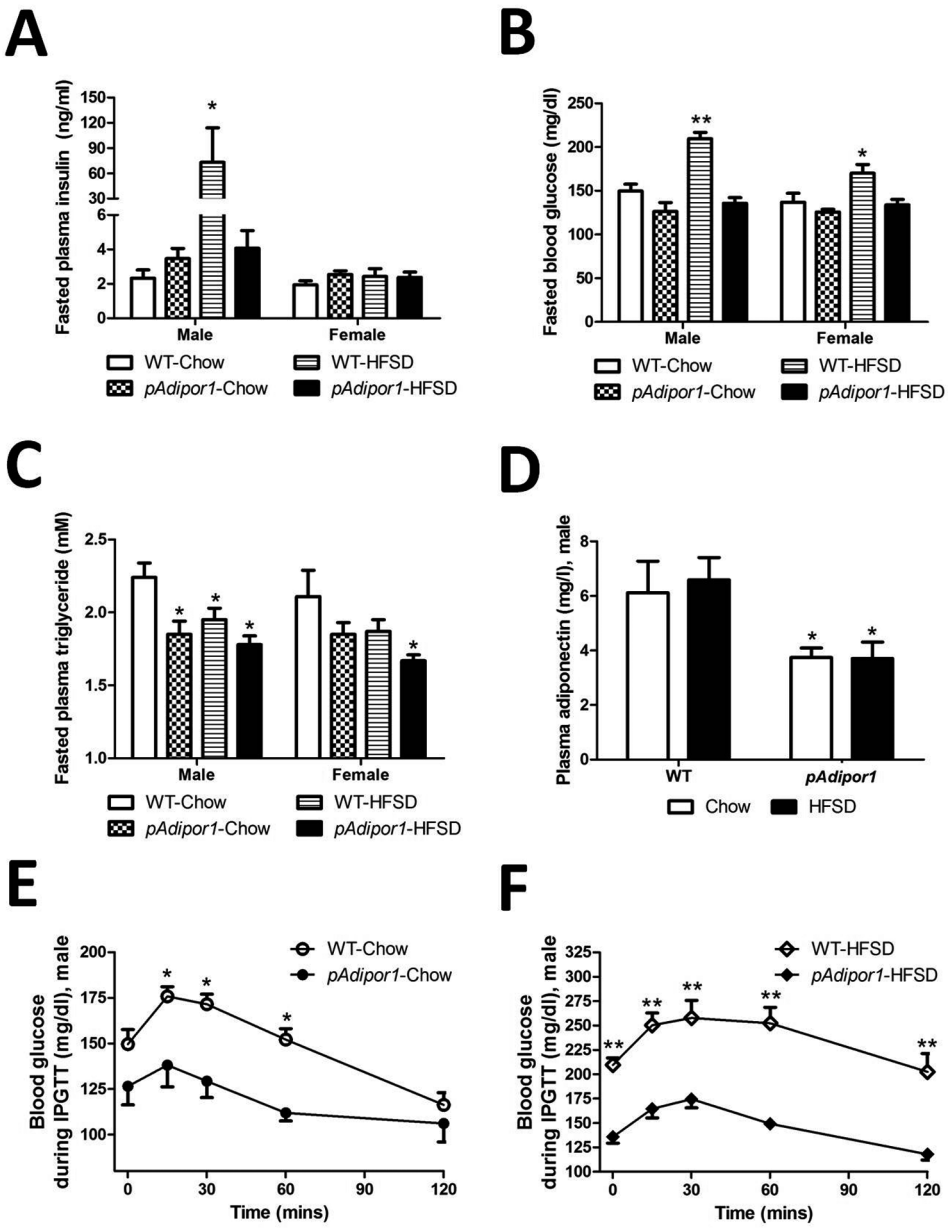

Fig. 2. Plasma values and intraperitoneal glucose tolerance test. The genetic and diet groups are as indicated in Fig. 1. There were 12 mice per genetic/diet/sex group. A: Fasted plasma insulin concentration. B: Fasted blood glucose concentration. C: Fasted plasma triglyceride concentration. D: Fasted plasma adiponectin concentration. Fasted insulin, triglyceride and adiponectin concentrations were measured after 25 weeks of feeding the diets to obviate the stress from the intraperitoneal glucose tolerance test. E: Intraperitoneal glucose tolerance test in male mice fed the Chow diets for 24 weeks. F: Intraperitoneal glucose tolerance test in male mice fed the HFSD diets for 24 weeks. All values were expressed as mean ±SEM. Differences among means for multiple comparisons were evaluated by two-way ANOVA and post-hoc test. Groups A−D were compared statistically to the control mice (WT-Chow). Groups E and F were compared statistically at each time point. $P \leq 0.05$: *, and $P \leq 0.01$: **.

2F), as evidenced by a substantial increase in the incremental glucose area under curve (AUC; WT-Chow: 17957 ± 49.2 mg/dl/2h, WT-HFSD: 28553 ± 57.4 mg/dl/2h. WT-Chow vs. WT-HFSD: $P \leq 0.001$). By contrast, male *pAdipor1* transgenic mice had better glucose tolerance when fed with either Chow or HFSD (*pAdipor1*-Chow: 14153 ± 24.5 mg/dl/2h, *pAdipor1*-HFSD: 17670 ± 27.9 mg/dl/2h in glucose AUC index, WT-HFSD vs.

pAdipor1-HFSD: $P \leq 0.001$, WT-HFSD vs. *pAdipor1*-Chow: $P \leq 0.001$, WT-Chow vs. *pAdipor1*-Chow: $P \leq 0.05$ and *pAdipor1*-Chow vs. *pAdipor1*-HFSD: $P \leq 0.05$). It should also be noted that *pAdipor1* transgenic mice had lower plasma glucose levels after a 14-h fast compared to male WT mice when fed with HFSD (Supplemental Fig. 2: refer to J-STAGE at https://www.jstage.jst.go.jp/browse/expanim), indicating an insulin-sensitizing effect for the *pAdipor1* transgene.

Effects of pAdipor1 transgene on the expression profile of metabolic genes in the adipose tissues of HFSD-fed mice

To explore the mechanisms underlying *pADIPOR1* action on HFSD treatment, we analyzed the expression profiles of metabolism-associated genes in metabolic tissues. Due to a more profound effect of *pAdipor1* transgene on HFSD-induced weight gain and plasma insulin level in the males, we chose the male mice to determine its effects on the expression profiles of genes in adipose tissue of HFSD-fed animals. Consistent with previous findings, HFSD sharply reduced adiponectin mRNA levels in WT mice (Fig. 3A). Adiponectin mRNA levels were lower in *pAdipor1* mice (compared to WT mice) and there was no effect of HFSD.

The insulin-sensitive glucose transporter 4 (*Glut4*) mRNA was decreased by HFSD in WT mice, whereas the *pAdipor1* transgene raised *Glut4* mRNAs in both the Chow and HFSD groups (Fig. 3B), suggesting increased glucose uptake that may lead to improve glucose tolerance in adipose tissue of HFSD-fed *pAdipor1* transgenic mice (Fig. 2E and F).

Peroxisome proliferator activated receptor alpha (*Pparα*) mRNA was increased in *pAdipor1* compared to WT mice with the effect being greater in the Chow-fed compared to the HFSD-fed mice (Fig. 3C). The acyl-CoA oxidase 1 (*Acox1*) mRNA was decreased in the HFSD-fed mice compared to Chow-fed mice regardless of genotype; the mRNA was increased in Chow-fed *pAdipor1* compared to WT mice (Fig. 3D). These results suggest an enhanced fatty acid oxidation in adipose tissue by the *pAdipor1* transgene. The *pAdipor1* transgene also prevented the down-regulation of fatty acids translocase (*CD36*) mRNA by HFSD (Fig. 3E). However, the expression of a *Pparα* target gene, mitochondrial uncoupling protein 2 (*Ucp2*), was not changed by either diet or genotype (Fig. 3F), suggesting that thermogenesis of the white adipose tissue is not the target of *pAdipor1* for the prevention of obesity and insulin resistance.

Effects of pAdipor1 transgene on the expression profile of metabolic genes in the skeletal muscles of HFSD-fed mice

In the skeletal muscles, we found that the expressions of genes associated with glucose uptake (Fig. 4A) and fatty acid oxidation (Fig. 4B, 4C and 4D) were decreased by HFSD in both WT and *pAdipor1* transgenic mice, except *Acox1* and carnitine palmitoyltransferase 1b (*Cpt1b*), suggesting that HFSD impaired the functions of glucose and lipid metabolism in the skeletal muscles. Moreover, *pAdipor1* increased *Pparα* mRNA, but decreased *Cpt1b* mRNA in Chow-fed mice (Fig. 4B and 4D). These findings suggest that *pAdipor1* transgene has the opposite effects on fatty acid oxidation and β-oxidation in the skeletal muscles of HFSD-fed mice. Moreover, HFSD had no effect in the gene expression of *CD36* (Fig. 4E) but increased the gene expression of *Ucp2* (Fig. 4F) in the skeletal muscles of both WT and *pAdipor1* transgenic mice, suggesting that thermogenesis was induced by HFSD.

Effects of pAdipor1 transgene on the expression profile of metabolic genes in the livers of HFSD-fed mice

In the liver, HFSD decreased gene expression of sterol regulatory element-binding transcription factor 1 (*Srebf1*) in WT, but not in *pAdipor1* mice (Fig. 5A). The *pAdipor1* transgene suppressed the *Srebf1* mRNA regardless of diet. For fatty acid synthase (*Fasn*), a *Srebf1* target gene, the *pAdipor1* transgene suppressed mRNA expression and HFSD further suppressed the mRNA, suggesting that liver lipogenesis was decreased by *Adipor1* (Fig. 5B). The *pAdipor1* transgene down regulated *Pparα* and *Acox1*, but not *Cpt1a* or *Ucp2* mRNA (Fig. 5C–F). The HFSD decreased *Pparα*, *Acox1* and *Cpt1a*, but not *Ucp2* mRNA in WT, but not in *pAdipor1* mice (Fig. 5C–F). These results suggest that the over-expressed *pAdipor1* has the opposite effect between skeletal muscle and liver under an HFSD challenge. As in adipose tissue, the expression of *Ucp2* gene in the liver was not affected by *pAdipor1* or HFSD (Fig. 5F).

pAdipor1 transgene prevented the HFSD-downregulated Pck1 mRNA

Expression of phosphoenolpyruvate carboxykinase 1 (*Pck1*) mRNA (PCK1 being a gluconeogenic and glyceroneogenic enzyme) in the liver was decreased by

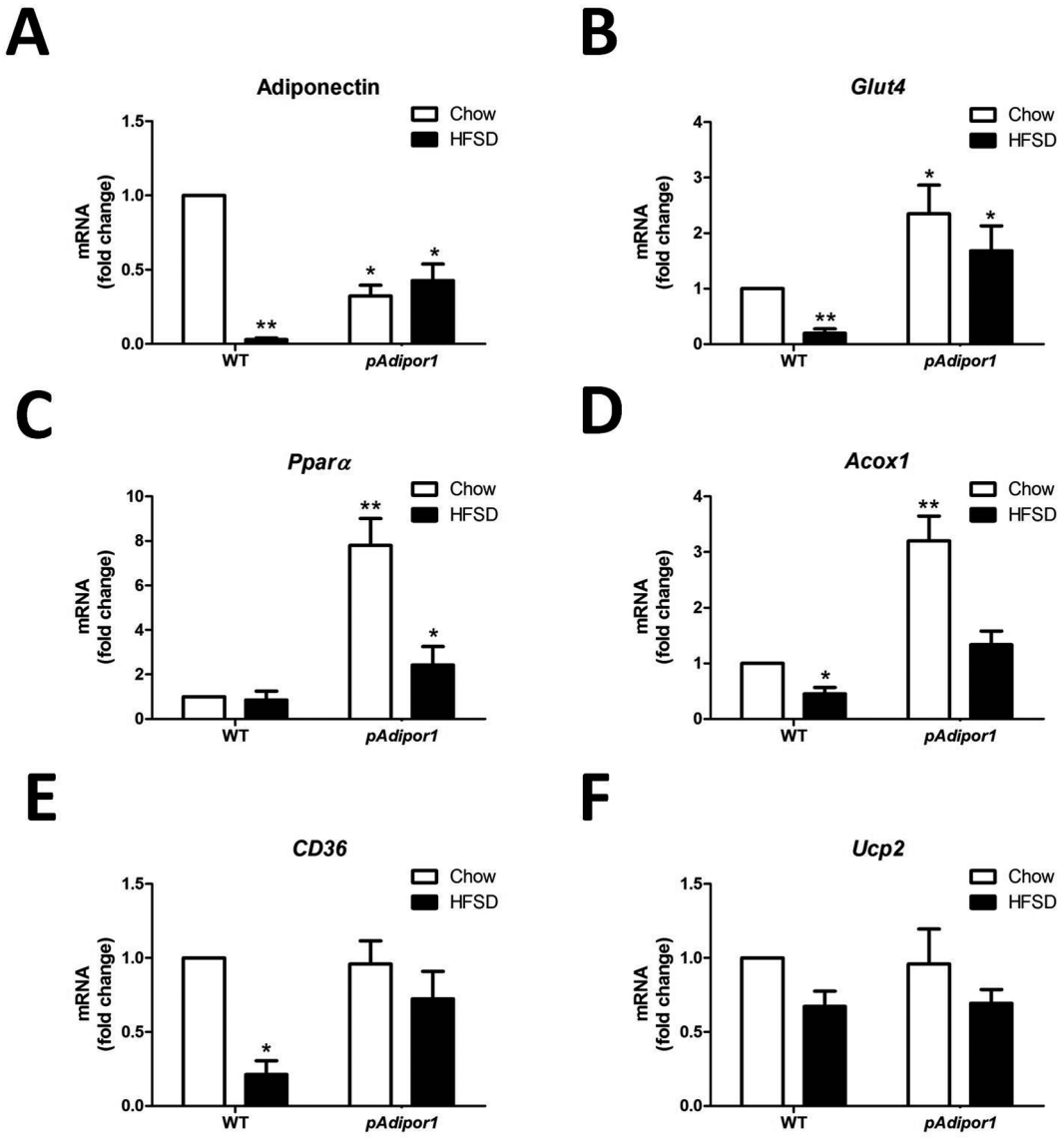

Fig. 3. The effects of the *pAdipor1* transgene on the expression profile of metabolic genes in the adipose tissue. The mice and diets were as indicated in Fig. 1. There are 12 mice per genetic/diet/sex group. Diets were fed for 25 weeks. A: Expression of adiponectin mRNA. B: Expression of *Glut4* mRNA. C: Expression of *Pparα* mRNA. D: Expression of *Acox1* mRNA. E: Expression of *CD36* mRNA. F: Expression of *Ucp2* mRNA. All values were expressed as mean ± SEM. Differences among means for multiple comparisons were evaluated by two-way ANOVA and post-hoc test. All groups were compared to the control mice (WT-Chow) for statistically significant differences. $P \leq 0.05$: *, and $P \leq 0.01$: **.

HFSD in WT, but not *pAdipor1* transgenic mice (Fig. 6A). It was decreased to an even greater extent in adipose tissue of HFSD-fed WT, but not in *pAdipor1* transgenic mice (Fig. 6B). Plasma glycerol levels were increased by HFSD in WT, but not in *pAdipor1* transgenic mice (Fig. 6C). The increased plasma glycerol levels and reduced *Pck1* mRNA in the liver and adipose tissue of WT suggests that the reesterification of triacylglycerol was dysregulated by HFSD, which can be ameliorated by *pAdipor1* transgene.

Discussion

Adiponectin has been recognized as an insulin-sensitizing adipokine that may have a role in preventing obesity and type II diabetes. Both adiponectin and its recep-

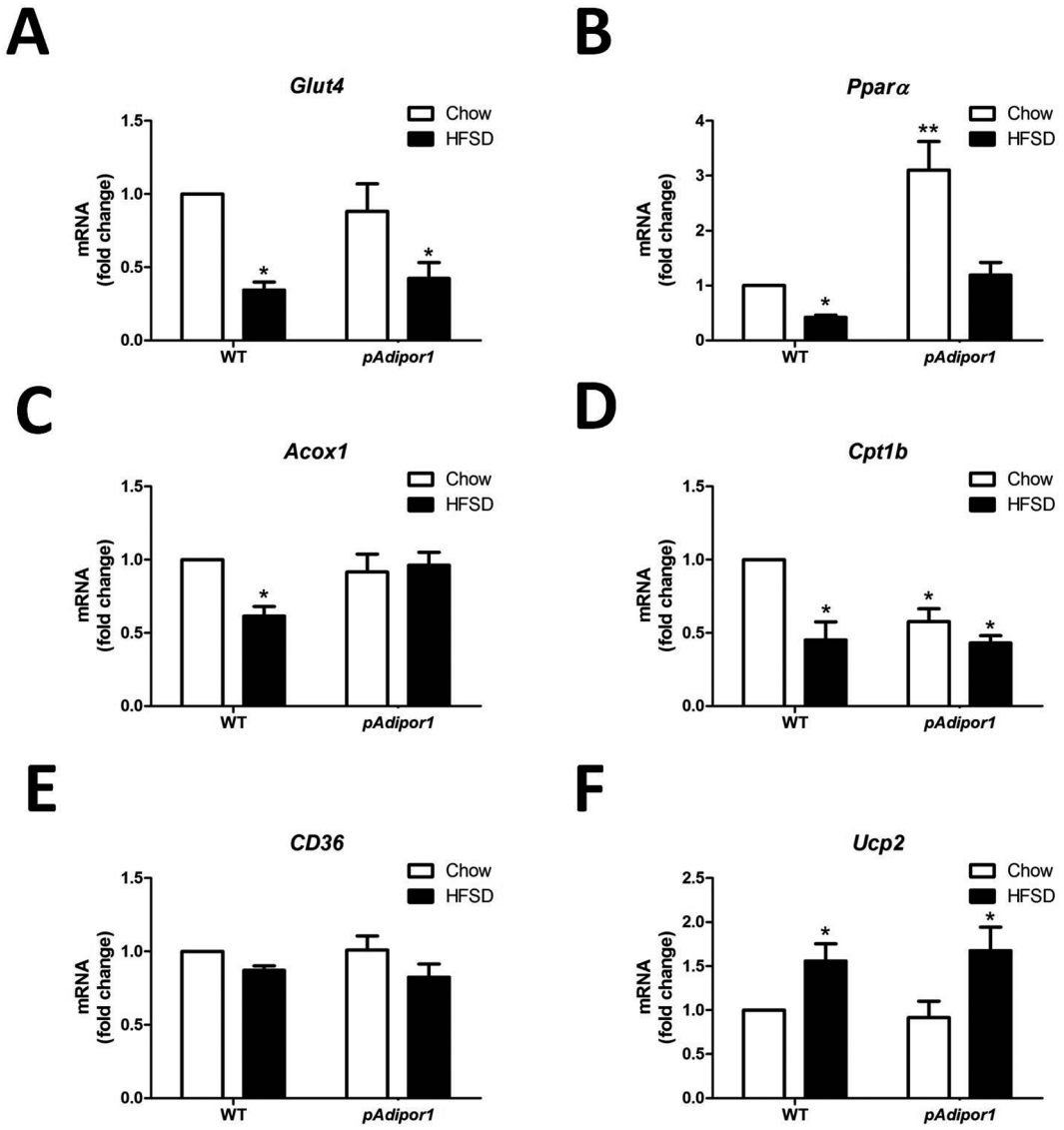

Fig. 4. The effects of the *pAdipor1* transgene on the expression profile of metabolic genes in the skeletal muscle. The mice and diets were as indicated in Fig. 1. There are 12 mice per genetic/diet/sex group. Diets were fed for 25 weeks. A: Expression of Glut4 mRNA. B: Expression of *Pparα* mRNA. C: Expression of *Acox1* mRNA. D: Expression of *Cpt1b* mRNA. E: Expression of *CD36* mRNA. F: Expression of *Ucp2* mRNA. All values were expressed as mean ± SEM. Differences among means for multiple comparisons were evaluated by two-way ANOVA and post-hoc test. All groups were compared to the control mice (WT-Chow) for statistically significant differences. $P \leq 0.05$: *, and $P \leq 0.01$: **.

tors are negatively associated with obesity and diabetes in human and rodent studies [2, 30, 40]. Our previous study indicated that *pAdipor1* is widely and consistently expressed in many tissues and to a greater extent than *pAdipor2*. Only one human study demonstrated that overexpression of *mAdipor1* in macrophages enhanced the actions of adiponectin to reduce body weight and fat accumulation [22]. However, the underlying mechanisms and variations between adiponectin and its receptors are still unclear in diet-induced obesity. In the current study, we hypothesized that *Adipor1* may have important regulatory functions when mice were fed HFSD. We generated *pAdipor1* transgenic mice and challenged them with HFSD to demonstrate that *Adipor1* can prevent diet-induced weight gain and hepatosteatosis.

The expression level of adiponectin is recognized to

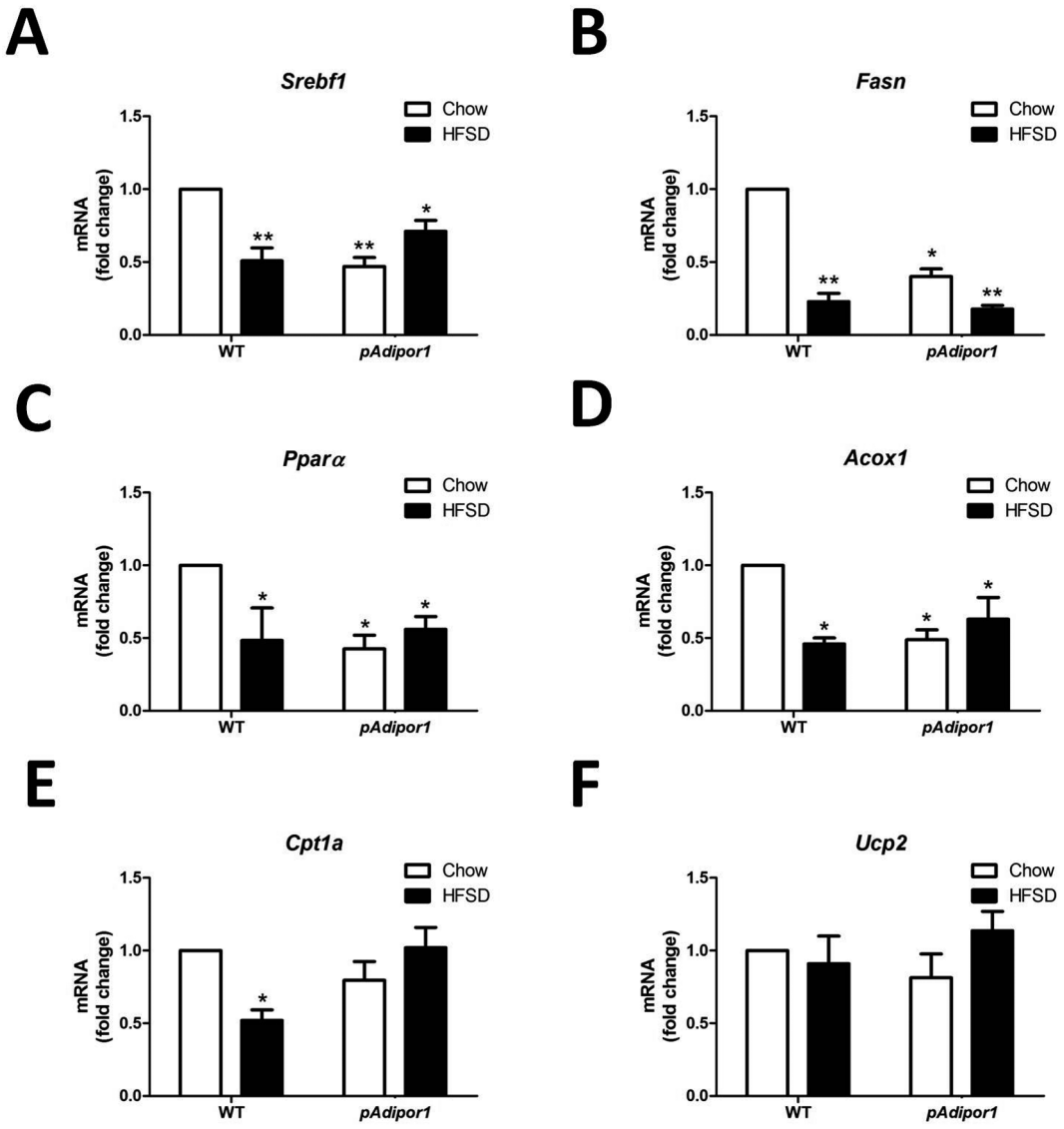

Fig. 5. The effects of the *pAdipor1* transgene on the expression profile of metabolic genes in the liver. The mice and diets were as indicated in Fig. 1. There are 12 mice per genetic/diet/sex group. Diets were fed for 25 weeks. A: Expression of *Srebf1* mRNA. B: Expression of *Fasn* mRNA. C: Expression of *Pparα* mRNA. D: Expression of *Acox1* mRNA. E: Expression of *Cpt1a* mRNA. F: Expression of *Ucp2* mRNA. All values were expressed as mean ± SEM. Differences among means for multiple comparisons were evaluated by two-way ANOVA and post-hoc test. All groups were compared to the control mice (WT-Chow) for statistically significant differences. $P \leq 0.05$: *, and $P \leq 0.01$: **.

negatively associate with obesity and diabetes [2]. Long-term (16 weeks) consumption of HFSD has been shown to increase circulating adiponectin, but decreases its mRNA levels in the adipose tissues of C57BL/6J mice and rats [7, 26, 44]. Similar effects were found in this study; when WT mice were fed HFSD (24 weeks), the adiponectin mRNA level in the adipose tissue was markedly reduced (Fig. 3A), but plasma adiponectin was maintained (Fig. 2D). Recent studies demonstrate that the actions of circulating adiponectin depend on which of the multiple active forms mediate energy homoeostasis [20]. Hence, the level of total plasma adiponectin is not a good marker to monitor the diet-induced obesity. *Adipor1* mRNA and the AMPK pathway in the muscle are decreased by HFSD, suggesting the inactivation of the signaling pathway for adiponectin [7]. Some studies indicated that there was the opposite, or no, effect on the expression of adiponectin and its receptors after HFSD

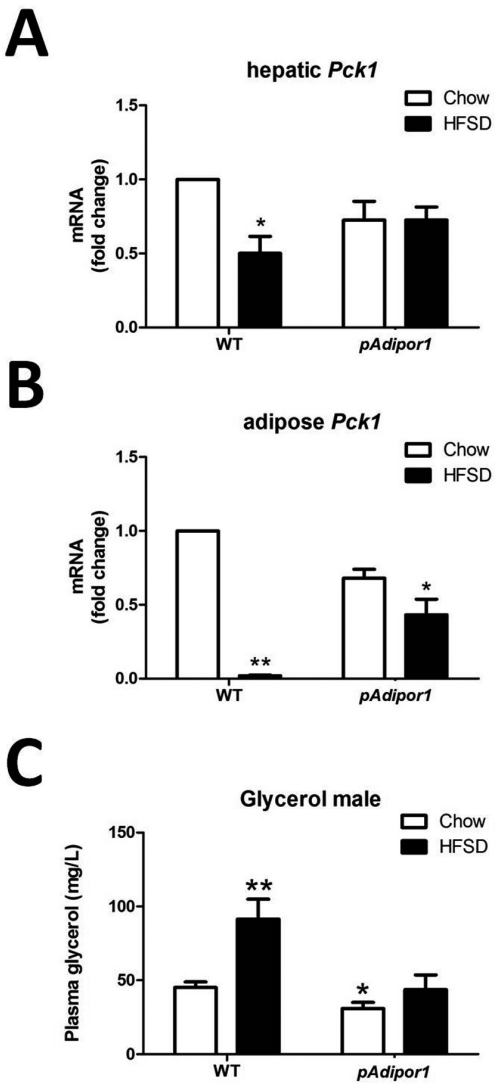

Fig. 6. The glyceroneogenesis of the *pAdipor1* transgene in the liver and adipose tissue. The genetic and diet groups are indicated in Fig. 1. There were 12 male mice per genetic/diet group. A: Expression of *Pck1* mRNA in the liver. B: Expression of *Pck1* mRNA in the adipose tissue. C: Plasma glycerol concentrations. All values were expressed as mean ± SEM. Differences among means for multiple comparisons were evaluated by two-way ANOVA and post-hoc test. All groups were compared to the control mice (WT-Chow) for statistically significant differences. $P \leq 0.05$: *, and $P \leq 0.01$: **.

feeding [8, 15]. These discrepancies may be due to the different treatment periods and extent of obesity or type II diabetes. We found that *pAdipor1* transgenic mice had decreased circulating adiponectin and mRNA in the adipose tissue. The *pAdipor1* transgene also reduced total *Adipor2* mRNA in the liver and muscle. These results suggest that there exists a negative feedback mechanism between adiponectin and its receptor. The *pAdipor1* transgene increased the membrane-bound ADIPOR1, but had no effects on the expression of *pAdipor1* mRNA in the liver and muscle (Supplemental Fig. 1B and 1C). This discrepancy suggested that the transgene product of ADIPOR1 may translocate quickly when synthesized in these tissues. Our findings that long-term feeding of HFSD resulted in weight gain, hepatosteatosis, glucose intolerance, hyperglycemia and hyperinsulinemia in FVB/N mice, but not in *pAdipor1* transgenic mice, suggest that the *pAdipor1* transgene improves glucose tolerance and prevents these symptoms. The *Adipor1*$^{-/-}$ mice have increased adiposity and decreased glucose tolerance [6]. In the current study, the *pAdipor1* transgenic mice were smaller than the WT mice even after long-term feeding of HFSD, suggesting that *pAdipor1* is involved in preventing weight gain and the metabolic syndrome.

Obesity and type II diabetes have been recognized to induce insulin resistance and disturb metabolic homeostasis. We found that the *pAdipor1* transgenic mice were smaller and had improved fatty acid oxidation associated genes expression in adipose tissue and skeletal muscle, but not in liver. Although there were opposite effects of fatty acid oxidation and fatty acid β-oxidation related genes expression in the liver and skeletal muscle of *pAdipor1* mice, the *pAdipor1* transgenic mice had reduced plasma triglyceride and maintained plasma glycerol concentration when fed with HFSD. The expression of genes associated with lipogenesis in the liver was also down-regulated by *pAdipor1* transgene and probably resulted in the lower level of plasma triglyceride. However, in the skeletal muscle, both glucose uptake and fatty acid β-oxidation associated genes expression were not up-regulated by the *pAdipor1* transgene. These results, suggest that the expression profile of metabolic genes in the adipose tissue of *pAdipor1* mice may play important roles in resisting diet-induced obesity.

ADIPOR1 activates the AMPK pathway and ADIPOR2 promotes the PPARα pathway in the liver of mice [43]. The same team further demonstrated that adiponectin and ADIPOR1 coordinated to activate peroxisome proliferator-activated receptor γ coactivator-1α (PGC1) in myocytes [17]. Hence, *pAdipor1* transgene increased the gene expression of *Pparα* in adipose tissue and skeletal muscle and may activate *via* the PGC1 pathway [23]. The induction of PGC1 and PPARα directly promoted

the expression and activity of glycerol kinase, which may control the homeostasis of triacylglycerol hydrolysis and fatty acid re-esterification in human adipocytes [23]. The HFSD increased plasma glycerol perhaps as a result of decreased triacylglycerol synthesis in liver and adipose tissue.

One of adiponectin functions is to decrease the expression of PCK1 and reduce hepatic gluconeogenesis in diabetic and WT mice [5, 9]. However, other evidences also indicate that glyceroneogenesis (the synthesis of glyceride-glycerol from sources other than glycerol and glucose) but not gluconeogenesis is the major action of PCK1 in the adipose tissue and is linked to diabetes and obesity [3, 4, 12, 24, 27]. Triacylglycerol turnover in the liver and adipose tissue affects the concentrations of plasma fatty acids and leads to glucose intolerance, insulin resistance and type II diabetes in mammals [28]. We found that the *pAdipor1* transgene only increased total *Adipor1* and *Adipor2* mRNA in the adipose tissue (Supplemental Fig. 1B). In the mice, the expression of *Adipor2* mRNA was highly expressed in the liver, not in the adipose tissue. Hence, the major action of PCK1 in our *pAdipor1* mice might act in the adipose tissue. Dysregulated glyceroneogenesis induces obesity, lipodystrophy, hepatosteatosis and type II diabetes in both *Pck1* gene-knockout and -overexpressing mice [12, 14, 29, 38]. Anti-diabetic PPARγ2 ligands increase the expression of *Pck1* mRNA and concomitantly increase the rate of glyceroneogenesis in adipose tissues [13, 37]. In the current study, we found that the expression of *Pck1* mRNA in the liver and adipose tissue were improved in *pAdipor1* mice fed with HFSD. Both adenovirus-mediated expression and targeted disruption of *Adipor1* indicate that *Adipor1* decreases the expression of *Pck1* and *Srebf1* mRNAs and leads to the inhibition of glucose production in the liver [43]. We found similar results in the liver and that the expression of *Glut4* mRNA was up-regulated in adipose tissue by the *pAdipor1* transgene, suggesting that *pAdipor1* was involved in moving glucose to the peripheral tissues. In brief, *pAdipor1* transgenic mice may increase *Pck1* and *Glut4* to promote glyceroneogenesis in adipose tissue to improve glucose tolerance.

In conclusion, our *pAdipor1* transgene prevented mice from developing diet-induced weight gain, hepatosteatosis and insulin resistance. The function of the overexpressed *pAdipor1* may be enhanced by maintaining high levels of *Pparα* mRNA in skeletal muscle and adipose tissue, and inhibiting the lipogenesis genes expression in liver. The *pAdipor1* may increase glyceroneogenesis as the result of up-regulation of *Pck1* and increase *Glut4* in the adipose tissue to increase glucose tolerance. These findings may lead to the development of novel therapeutic strategies for treating metabolic syndromes and obesity.

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