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

Bing-Hsien LIU1, Yuan-Yu LIN1, Ya-Chin WANG1, Chao-Wei HUANG1, Chih-Chien CHEN1, Shinn-Chih WU1,2, Harry J. MERSMANN1, Winston T.K. CHENG1,3, and Shih-Torng DING1,2

1) Department of Animal Science and Technology, National Taiwan University, No. 50, Ln. 155, Sec. 3, Keelung Rd., Da’an Dist., Taipei City 106, Taiwan
2) Institute of Biotechnology, National Taiwan University, No. 81, Changxing St., Da’an Dist., Taipei City 106, Taiwan
3) Department of Animal Science and Biotechnology, Tunghai University, No. 1727, Sec. 4, Taiwan Blvd., Xitun Dist., Taichung City 407, Taiwan

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
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.

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). All experiments were carried out on both male and female mice with homologous offspring from the F3 or later generations (n=6 for each line/sex).

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

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
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-tribromoethanol (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 CO₂ 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).

Table 1. Primer sets for qPCR

| Gene name | Primers 5’-3’ (forward and reverse) | Length (bp) | Annealing temperature (°C) | Reference sequence |
|-----------|------------------------------------|-------------|--------------------------|------------------|
| DsRed2    | F: GACCCACACGGCCCTGAAG, R: TGGCCTCCAGTGTTGAGTCC | 159         | 64                       | EU016077         |
| Adiponectin| F: GGGCTCTGTGCTGCTCACTC, R: AGAGTCTGGTACGTATCTGCATAG | 101         | 55                       | NM_009605        |
| Adipor1   | F: CCTGGCTCTATTACCTCTCTC, R: GAAACACTTGTGCTTTGTTCT | 149         | 64                       | NM_011144        |
| Ppara     | F: TGCTGGATCGGCTCAATAA, R: TCCTGCCACTTCTGCTAC | 114         | 64                       | NM_011144        |
| Acox1     | F: AGTTCACACTGCAGGCAAT, R: GAGTGCTTTGACCTCCTGAT | 81          | 64                       | NM_015729        |
| Cpt1a     | F: GTTCTCAGTATAGGCCATG, R: GAATACCAACAGGGTGC | 102         | 62                       | BC054791         |
| Cpt1b     | F: TTTGGGAAACCACACCTCCGCAA, R: TTTGCTCTGTGAGCCTGCA | 262         | 60                       | NM_009948        |
| CD36      | F: CAAGCTCTTGGGCATGGTAGA, R: TGGATTGGCAGCAACATATGAA | 92          | 64                       | NM_007643        |
| Ucp2      | F: CTCTTCTCTGGGAGCCAATC, R: CCCCCTCACCTTCTTAGCAG | 99          | 62                       | NM_011671        |
| Srebf1    | F: GAACCACGGGTGGGAACACAGGC, R: GACGCGGCGAGCTGGGTTTCT | 224         | 57                       | NM_011480        |
| Fasn      | F: GGGCAGCTGACTGCTGTTTCC, R: GGATCGAGGAAGGATCAAGAGC | 200         | 60                       | NM_007988        |
| Glut4     | F: TACATATCTGACAGGCCAAGG, R: TCGGTGTTTGGACACCGCTTCC | 131         | 58                       | NM_009204        |
| Pck1      | F: GTCCACCATCCTCTTGGAAGA, R: GGTCGCAATCGCGAGGTG | 174         | 64                       | NM_011044        |
| β-actin   | F: TGTTCCCAGGTGGGCCATGC, R: CTTTCAAGGTGGGCCATAG | 130         | 62                       | NM_007393        |
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 embedded 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 pAdipo1 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

*pAdipo1* transgenic mice were small and resistant to HFSD-induced hepatosteatosis

The *pAdipo1* 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 *Adipo1* and *Adipo2* mRNA levels were much greater than in other tissues (Supplemental Fig. 1B). Total *Adipo2* mRNA in liver and muscle were depressed. Consistent with its role in modulating energy homeostasis, we found that the *pAdipo1* 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 *pAdipo1* 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 *pAdipo1* transgenic mice (Fig. 1C; male), confirming the beneficial effects of *pAdipo1* transgene on hepatosteatosis.

Glucose intolerance is not seen in HFSD-fed *pAdipo1* 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 *pAdipo1* 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 *pAdipo1* transgenic mice, confirming an anti-diabetic role for *pAdipo1* transgene (Fig. 2A and 2B).

Plasma triglyceride levels (Fig. 2C) were lower in *pAdipo1* transgenic male mice than in WT male mice when fed Chow. HFSD lowered plasma triglyceride in male WT mice and in female *pAdipo1* transgenic mice. These results suggest that the male mice were more sensitive to the HFSD challenge and had poorer glucose
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 downregulated in \textit{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...
2F), as evidenced by a substantial increase in the incre-
mental 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 ≤ 0.001). By contrast,

male pAdipor1 transgenic mice had better glucose tol-
erance when fed with either Chow or HFSF (pAdipor1-
Chow: 14153 ± 24.5 mg/dl/2h, pAdipor1-HFSD: 17670 ± 27.9 mg/dl/2h in glucose AUC index, WT-HFSD vs.
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 transgenic mice (Fig. 2E and F). The HFSD decreased adiponectin mRNA expression and HFSD further suppressed the mrna, suggesting that thermogenesis was induced by HFSD.

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 Ppara 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 Ppara and Acox1, but not Cpt1a or Ucp2 mRNA (Fig. 5C–F). The HFSD decreased Ppara, 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 glycogenetic enzyme) in the liver was decreased by
HFSD in WT, but not \textit{pAdipor1} transgenic mice (Fig. 6A). It was decreased to an even greater extent in adipose tissue of HFSD-fed WT, but not in \textit{pAdipor1} transgenic mice (Fig. 6B). Plasma glycerol levels were increased by HFSD in WT, but not in \textit{pAdipor1} transgenic mice (Fig. 6C). The increased plasma glycerol levels and reduced \textit{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 \textit{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-
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. 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 Ppara 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≤0.05: *, and P≤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 homeostasis [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. 5. The effects of the pAdiport1 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 Srebfl mRNA. B: Expression of Fasn mRNA. C: Expression of Ppara 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 $P \leq 0.01$: **.
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 re-
sults suggest that there exists a negative feedback mech-
nanism between adiponectin and its receptor. The $pAdi-
por1$ transgene increased the membrane-bound
ADIPOR1, but had no effects on the expression of $pA-
dipor1$ 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, hepatos-
teatosis, glucose intolerance, hyperglycemia and hyper-
insulinemia in FVB/N mice, but not in $pAdipor1$
transgenic mice, suggest that the $pAdipor1$ transgene
improves glucose tolerance and prevents these symp-
toms. 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 homeo-
stasis. We found that the $pAdipor1$ transgenic mice were
smaller and had improved fatty acid oxidation associ-
ated 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 glyc-
erol 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. How-
ever, in the skeletal muscle, both glucose uptake and
fatty acid β-oxidation associated genes expression were
not up-regulated by the $pAdipor1$ transgene. These re-
sults, 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 ADI-
POR2 promotes the PPARα pathway in the liver of mice
[43]. The same team further demonstrated that adipo-
nectin and ADIPOR1 coordinated to activate peroxisome
proliferator-activated receptor γ coactivator-1α (PGC1)
in myocytes [17]. Hence, $pAdipor1$ transgene increased
the gene expression of $Ppara$ in adipose tissue and skel-
etal muscle and may activate via the PGC1 pathway [23].
The induction of PGC1 and PPARα directly promoted

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 concentra-
tions. 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 $P\leq 0.01$: **.
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 Ppara 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.

**Acknowledgments**

The authors would like to express our gratitude to the lab members for their help and input during the study. The study was supported in part by grants from the National Science Council in Taiwan. C.-C. CHEN was supported by the postdoctoral fellowship grant (NSC099-2811-B-029-003) from the National Science Counsel in Taiwan.

**References**

1. Bartels, E.D., Bang, C.A., and Nielsen, L.B. 2009. Early atherosclerosis and vascular inflammation in mice with diet-induced type 2 diabetes. *Eur. J. Clin. Invest.* 39: 190–199. [Medline] [CrossRef]

2. Bauer, S., Weigert, J., Neumeier, M., Wanninger, J., Schaffer, A., Luchner, A., Schnitzbauer, A.A., Aslanidis, C., and Buechler, C. 2010. Low-abundant adiponectin receptors in visceral adipose tissue of humans and rats are further reduced in diabetic animals. *Arch. Med. Res.* 41: 75–82. [Medline] [CrossRef]

3. Beale, E.G., Harvey, B.J., and Forest, C. 2007. PCK1 and PCK2 as candidate diabetes and obesity genes. *Cell Biochem. Biophys.* 48: 89–95. [Medline] [CrossRef]

4. Beale, E.G., Hammer, R.E., Antoine, B., and Forest, C. 2004. Disregulated glyceroneogenesis: PCK1 as a candidate diabetes and obesity gene. *Trends Endocrinol. Metab.* 15: 129–135. [Medline] [CrossRef]

5. Berg, A.H., Combs, T.P., Du, X., Brownlee, M., and Scherer, P.E. 2001. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat. Med.* 7: 947–953. [Medline] [CrossRef]

6. Bjursell, M., Ahnmark, A., Bohlooly, Y.M., William-Olsson, L., Rhedin, M., Peng, X.R., Ploj, K., Gerdin, A.K., Arnerup, G., Elmgren, A., Berg, A.L., Oscarsson, J., and Linden, D. 2007. Opposing effects of adiponectin receptors 1 and 2 on energy metabolism. *Diabetes* 56: 583–593. [Medline] [CrossRef]

7. Bonnard, C., Durand, A., Vidal, H., and Rieuxset, J. 2008. Changes in adiponectin, its receptors and AMPK activity in tissues of diet-induced diabetic mice. *Diabetes Metab.* 34: 52–61. [Medline] [CrossRef]

8. Bullen, J.W. Jr., Bluhé, S., Kelesidis, T., and Mantzoros, C.S. 2007. Regulation of adiponectin and its receptors in response to development of diet-induced obesity in mice. *Am. J. Physiol. Endocrinol. Metab.* 292: E1079–E1086. [Med-
ADIPONECTIN RECEPTOR1, METABOLIC SYNDROMES

line] [CrossRef]
9. Combs, T.P., Berg, A.H., Obici, S., Scherer, P.E., and Rossetti, L. 2001. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. J. Clin. Invest. 108: 1875–1881. [Medline] [CrossRef]
10. Ding, S.T., Liu, B.H., and Ko, Y.H. 2004. Cloning and expression of porcine adiponectin and adiponectin receptor 1 and 2 genes in pigs. J. Anim. Sci. 82: 3162–3174. [Medline] [CrossRef]
11. Fantuzzi, G. 2005. Adipose tissue, adipokines, and inflammation. J. Allergy Clin. Immunol. 115: 911–919, quiz 920. [Medline] [CrossRef]
12. Franckhauser, S., Munoz, S., Pujol, A., Casellas, A., Riu, E., Otaegui, P., Su, B., and Bosch, F. 2002. Increased fatty acid re-esterification by PEPCK overexpression in adipose tissue leads to obesity without insulin resistance. Diabetes 51: 624–630. [Medline] [CrossRef]
13. Glorian, M., Duplus, E., Beale, E.G., Scott, D.K., Granner, D.K., and Forest, C. 2001. A single element in the phosphoenolpyruvate carboxykinase gene mediates thiazolidinedione action specifically in adipocytes. Biochimie 83: 933–943. [Medline] [CrossRef]
14. Hakimi, P., Johnson, M.T., Yang, J., Lepage, D.F., Conlon, R.A., Kalhan, S.C., Reshef, L., Tilghman, S.M., and Hanson, R.W. 2005. Phosphoenolpyruvate carboxykinase and the critical role of capatalase in the control of hepatic metabolism. Nutr. Metab. (Lond) 2: 33. [Medline] [CrossRef]
15. Harada, K., Shen, W.J., Patel, S., Natu, V., Wang, J., Osuga, J., Ishibashi, S., and Kraemer, F.B. 2003. Resistance to high-fat diet-induced obesity and altered expression of adipose-specific genes in HSL-deficient mice. Am. J. Physiol. Endocrinol. Metab. 285: E1182–E1195. [Medline] [CrossRef]
16. Hotamisligil, G.S., Shargill, N.S., and Spiegelman, B.M. 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259: 87–91. [Medline] [CrossRef]
17. Iwabu, M., Yamauchi, T., Okada-Iwabu, M., Sato, K., Nakagawa, T., Funata, M., Yamaguchi, M., Namiki, S., Nakayama, R., Tabata, M., Ogata, H., Kubota, N., Takamoto, I., Hayashi, Y.K., Yamauchi, N., Waki, H., Fukayama, M., Nishino, I., Tokuyama, K., Ueki, K., Oike, Y., Ishii, S., Hirose, K., Shimizu, T., Touhara, K., and Kadowaki, T. 2010. Adiponectin and AdipoR1 regulate PGC-1alpha and mitochondrial dysfunction by Ca(2+) and AMPK/SIRT1. Nature 464: 1313–1319. [Medline] [CrossRef]
18. Liu, B.H., Wang, P.H., Wang, Y.C., Cheng, W.M., Mersmann, H.J., and Ding, S.T. 2008. Fasting regulates the expression of adiponectin receptors in young growing pigs. J. Anim. Sci. 86: 3377–3384. [Medline] [CrossRef]
19. Liu, B.H., Wang, Y.C., Wu, S.C., Mersmann, H.J., Cheng, W.T., and Ding, S.T. 2008. Insulin regulates the expression of adiponectin and adiponectin receptors in porcine adipocytes. Domest. Anim. Endocrinol. 34: 352–359. [Medline] [CrossRef]
20. Liu, M., and Liu, F. 2010. Transcriptional and post-translational regulation of adiponectin. Biochem. J. 425: 41–52. [Medline] [CrossRef]
21. Livet, J., Weissman, T.A., Kang, H., Draft, R.W., Lu, J., Ben-ness, R.A., Sanes, J.R., and Lichtman, J.W. 2007. Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. Nature 450: 56–62. [Medline] [CrossRef]
22. Luo, N., Chung, B.H., Wang, X., Klein, R.L., Tang, C.K., Garvey, W.T., and Fu, Y. 2013. Enhanced adiponectin actions by overexpression of adiponectin receptor 1 in macrophages. Atherosclerosis 228: 124–135. [Medline] [CrossRef]
23. Mazzucchelli, A., Viguier, N., Tiraby, C., Annicotte, J.S., Mairal, A., Klimcakova, E., Lepin, E., Delmar, P., Dejean, S., Tavernier, G., Lefort, C., Hidalgo, J., Pineau, F., Fajas, L., Clement, K., and Langin, D. 2007. The transcriptional co-activator peroxisome proliferator activated receptor (PPAR) gamma coactivator-1 alpha and the nuclear receptor PPAR alpha control the expression of glycerol kinase and metabolism genes independently of PPAR gamma activation in human white adipocytes. Diabetes 56: 2467–2475. [Medline] [CrossRef]
24. Millward, C.A., Desantis, D., Hsieh, C.W., Heaney, J.D., Pisanu, S., Olswang, Y., Reshef, L., Beidelschies, M., Puchowicz, M., and Croniger, C.M. 2010. Phosphoenolpyruvate carboxykinase (Pck1) helps regulate the triglyceride/fatty acid cycle and development of insulin resistance in mice. J. Lipid Res. 51: 1452–1463. [Medline] [CrossRef]
25. Montgomery, M.K., Hallahan, N.L., Brown, S.H., Liu, M., Mitchell, T.W., Cooney, G.J., and Turner, N. 2013. Mouse strain-dependent variation in obesity and glucose homeostasis in response to high-fat feeding. Diabetologia 56: 1129–1139. [Medline] [CrossRef]
26. Naderali, E.K., Estadella, D., Rocha, M., Pickavance, L.C., Fatani, S., Denis, R.G., and Williams, G. 2003. A fat-enriched, glucose-enriched diet markedly attenuates adiponectin mRNA levels in rat epididymal adipose tissue. Clin. Sci. (Lond) 105: 403–408. [Medline] [CrossRef]
27. Nye, C.K., Hanson, R.W., and Kalhan, S.C. 2008. Glycero- neogenesis is the dominant pathway for triglyceride glycerol synthesis in vivo in the rat. J. Biol. Chem. 283: 27565–27574. [Medline] [CrossRef]
28. Nye, C., Kim, J., Kalhan, S.C., and Hanson, R.W. 2008. Reassessing triglyceride synthesis in adipose tissue. Trends Endocrinol. Metab. 19: 356–361. [Medline] [CrossRef]
29. Olswang, Y., Cohen, H., Papo, O., Cassuto, H., Croniger, C.M., Hakimi, P., Tilghman, S.M., Hanson, R.W., and Reshef, L. 2002. A mutation in the peroxisome proliferator-activated receptor gamma-binding site in the gene for the cystolic form of phosphoenolpyruvate carboxykinase reduces adipose tissue size and fat content in mice. Proc. Natl. Acad. Sci. U.S.A. 99: 625–630. [Medline] [CrossRef]
30. Peng, Y., Rideout, D., Rakita, S., Sajan, M., Fares, R., You, M., and Murr, M.M. 2009. Downregulation of adiponectin/AdipoR2 is associated with steatohepatitis in obese mice. J. Gastrointest. Surg. 13: 2043–2049. [Medline] [CrossRef]
31. Ramirez-Zacarias, J.L., Castro-Munozledo, F., and Kuri-Harcuch, W. 1992. Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O. Histochemistry 97: 493–497. [Medline] [CrossRef]
32. Schmid, R.S., Yokota, Y., and Anton, E.S. 2006. Generation and characterization of brain lipid-binding protein promoter-based transgenic mouse models for the study of radial glia.
33. Schreier, S.A., Wilson, D.L., and LeBoeuf, R.C. 1998. C57BL/6 mice fed high fat diets as models for diabetes-accelerated atherosclerosis. Atherosclerosis 136: 17–24. [Medline] [CrossRef]

34. Shitara, H., Sato, A., Hayashi, J., Mizushima, N., Yonekawa, H., and Taya, C. 2004. Simple method of zygosity identification in transgenic mice by real-time quantitative PCR. Transgenic Res. 13: 191–194. [Medline] [CrossRef]

35. Sone, H. and Kagawa, Y. 2005. Pancreatic beta cell senescence contributes to the pathogenesis of type 2 diabetes in high-fat diet-induced diabetic mice. Diabetologia 48: 58–67. [Medline] [CrossRef]

36. Sumiyoshi, M., Sakanaka, M., and Kimura, Y. 2006. Chronic intake of high-fat and high-sucrose diets differentially affects glucose intolerance in mice. J. Nutr. 136: 582–587. [Medline]

37. Tordjman, J., Chauvet, G., Quette, J., Beale, E.G., Forest, C., and Antoine, B. 2003. Thiazolidinediones block fatty acid release by inducing glyceroneogenesis in fat cells. J. Biol. Chem. 278: 18785–18790. [Medline] [CrossRef]

38. Valera, A., Pujol, A., Pelegrin, M., and Bosch, F. 1994. Transgenic mice overexpressing phosphoenolpyruvate carboxykinase develop non-insulin-dependent diabetes mellitus. Proc. Natl. Acad. Sci. U.S.A. 91: 9151–9154. [Medline] [CrossRef]

39. Vintersten, K., Monetti, C., Gertsenstein, M., Zhang, P., Laszlo, L., Biechele, S., and Nagy, A. 2004. Mouse in red: red fluorescent protein expression in mouse ES cells, embryos, and adult animals. Genesis 40: 241–246. [Medline] [CrossRef]

40. Wade, T.E., Mathur, A., Lu, D., Swartz-Basile, D.A., Pitt, H.A., and Zyromski, N.J. 2009. Adiponectin receptor-1 expression is decreased in the pancreas of obese mice. J. Surg. Res. 154: 78–84. [Medline] [CrossRef]

41. Wang, Y., Lam, K.S., Chan, L., Chan, K.W., Lam, J.B., Lam, M.C., Hoo, R.C., Mak, W.W., Cooper, G.J., and Xu, A. 2006. Post-translational modifications of the four conserved lysine residues within the collagenous domain of adiponectin are required for the formation of its high molecular weight oligomeric complex. J. Biol. Chem. 281: 16391–16400. [Medline] [CrossRef]

42. Wellen, K.E. and Hotamisligil, G.S. 2005. Inflammation, stress, and diabetes. J. Clin. Invest. 115: 1111–1119. [Medline]

43. Yamauchi, T., Nio, Y., Maki, T., Kobayashi, M., Takazawa, T., Iwabu, M., Okada-Iwabu, M., Kawamoto, S., Kubota, N., Kubota, T., Ito, Y., Kamon, J., Tsuchida, A., Kumagai, K., Kozono, H., Hada, Y., Ogata, H., Tokuyama, K., Tsunoda, M., Ide, T., Murakami, K., Awazawa, M., Takamoto, I., Froguel, P., Hara, K., Tobe, K., Nagai, R., Ueki, K., and Kadowaki, T. 2007. Targeted disruption of Adipor1 and Adipor2 causes abrogation of adiponectin binding and metabolic actions. Nat. Med. 13: 332–339. [Medline] [CrossRef]

44. Yang, B., Chen, L., Qian, Y., Triantafillou, J.A., McNulty, J.A., Carrick, K., Clifton, L.G., Han, B., Geske, R., Strum, J., Brown, K.K., Stimpson, S.A., and Pahel, G. 2006. Changes of skeletal muscle adiponectin content in diet-induced insulin resistant rats. Biochem. Biophys. Res. Commun. 341: 209–217. [Medline] [CrossRef]