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The function of porcine PPARγ and dietary fish oil effect on the expression of lipid and glucose metabolism related genes☆

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Abstract

Peroxisome-proliferator-activated receptor γ (PPARγ) plays a critical role in regulation of adipocyte differentiation and insulin sensitivity. To become functional, PPARγ must be activated by binding an appropriate ligand. Polyunsaturated fatty acids (PUFA) are potential ligands for PPARγ. The current experiment was designed to determine the potential for PUFA, particularly eicosapentaenoic acid and docosahexaenoic acid, to activate the function of porcine PPARγ in vivo. Transgenic mice, expressing porcine PPARγ in skeletal muscle were generated and fed with a high-saturated fat (beef tallow) or high-unsaturated fat (fish oil) diet for 4 months. When transgenic mice were fed a fish oil supplemented diet, the expression of adipogenic and glucose uptake genes was increased, leading to reduced plasma glucose concentration. The PPARγ transgene increased the expression of Glut4 in the muscle. This result suggests that there was increased glucose utilization and, therefore, a reduced blood glucose concentration in the transgenic mice. Also, the plasma adiponectin was elevated by fish oil treatment, suggesting a role of adiponectin in mediating the PUFA effect. These results suggest that PUFA may serve as a natural regulator of glucose uptake in vivo and these effects are mainly through PPARγ function.

Keywords: Adiponectin; Fish oil; Insulin sensitivity; Peroxisome proliferator-activated receptor γ; Polyunsaturated fatty acids; Mice

1. Introduction

Adipocyte differentiation is complicated and regulated by several transcription factors, including CCAAT/enhancer-binding protein α, peroxisome proliferator-activated receptor γ (PPARγ) and sterol regulatory element binding protein-1c (SREBP-1c or ADD1). Generally, PPARγ is considered to be the most important transcription factor in regulation of adipocyte differentiation [1]. In addition, PPARγ also plays a critical role in maintenance of whole-body glucose homeostasis [2].

For activation of PPAR, a ligand needs to bind to its ligand binding domain. Polyunsaturated fatty acids (PUFA) such as arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have high binding affinity for PPARγ [3–5]. In the past decades, it has been demonstrated that the arachidonate metabolite, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, is a possible endogenous ligand for PPARγ [6]. Putative metabolites of DHA also are PPARγ activators [7]. These results suggest that PUFA and PUFA metabolites are able to regulate PPAR activity. We demonstrated that DHA treatment increases PPARγ-responsive gene expression in a cell model [8]. Plasma adiponectin is elevated when mice are fed fish oil [9]. Adiponectin is involved in insulin sensitivity and its gene is PPARγ-responsive. These results imply that the enhancement of insulin sensitivity by PUFA is mediated by activation of PPARγ and PUFA may be endogenous ligands for PPARγ. However, most research demonstrating PPARγ ligand activity uses in vitro cell models. No direct evidence is available to indicate that PUFA is able to activate PPARγ in vivo [10].

In previous studies, we demonstrated that porcine PPARγ and PPARδ regulate lipid metabolism [8,11]. The PUFA, DHA or its metabolites are able to activate porcine PPARγ [12]. Because muscle is the major organ to metabolize glucose and one of the important organs to utilize lipids, we generated muscle-specific expression of porcine PPARγ in transgenic mice to study the function of PPARγ. Feeding experiments supplemented with either fish oil or PPARγ ligands was carried out to demonstrate whether the fish oil can mimic PPARγ ligands in vivo.

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2. Materials and methods

2.1. Generation of transgenic mice

For construction of skeletal muscle-specific expression of porcine PPARγ, the porcine PPARγ cDNA was under the transcriptional regulation of the mouse 1.8 kb mouse myoblast determination protein-1 (MyoD) promoter/enhancer and polyadenylated by the SV40 polyadenylation sequences (Fig. 1A). It has been demonstrated that this promoter sequence is functional and regulated by myoblast-specific transcription factors [13]. The porcine PPARγ sequence combined with the SV40 polyadenylation sequences was successfully cloned in previous studies [11]. Transgenic mice were generated by microinjection of the transgene into pronuclei of fertilized FVB/NJ mouse embryos. Founder mouse tail genomic DNA was extracted and the presence of porcine PPARγ was confirmed by polymerase chain reaction (PCR) and Southern blot techniques. Southern blots were prepared following a standard protocol [14]. Skeletal muscle PPARγ protein from transgenic mice was also identified by standard western blot with modifications described in our previous study [15]. The anti-PPARγ polyclonal antibody (sc-1984; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used to determine the expression of the transgene. Positive transgenic progenies were backcrossed with FVB/NJ to generate homogenous transgenic mice. Transgenic mice from the F3 generation were used in this study.

2.2. Animal experiments

In the diet study, wild-type (FVB/NJ) and PPARγ transgenic mice were age- (7–8 weeks old) and gender-matched (male mice). The animal protocol was approved by the Animal Care and Use Committee of the National Taiwan University. Wild-type and PPARγ transgenic mice were each assigned to four groups (n=10 in each group). The first group was fed a standard diet, that on a caloric basis, contained 58% carbohydrate, 13.5% fat and 28.5% protein (LabDiet 5001, LabDiet, Richmond, IN, USA). The second group was fed a customized rosiglitazone-containing (4 mg/kg: Cayman Chemicals, Ann Arbor, MI, USA) standard diet, that contained, on a caloric basis, 58% carbohydrate, 13.5% fat and 28.5% protein (Bioserv, Frenchtown, NJ, USA). The third group was fed a high-fish-oil (mainly from tuna containing 7% EPA and 24% DHA) diet, that on a caloric basis, contained 36% carbohydrate, 35.5% fat and 28.5% protein (Bioserv). The fourth group was fed a high-beef tallow diet, that on a caloric basis, contained 36% carbohydrate, 35.5% fat and 28.5% protein (Bioserv). Mice were fed each diet for four months. Feed intake was determined monthly. After 4 months, mice were weighed and sacrificed by cervical dislocation with anesthesia (tribromoethanol, 0.4 mg/g i.p. of body weight; Sigma, St. Louis, MO, USA). Whole white adipose tissue (epididymal fat in males and ovarian/uterine fat in females) was isolated immediately and weighed. Liver (right lobe) and white adipose tissue were fixed for histochemical staining. Liver and skeletal muscles (extensor digitorum longus) were frozen in liquid nitrogen for RNA extraction.

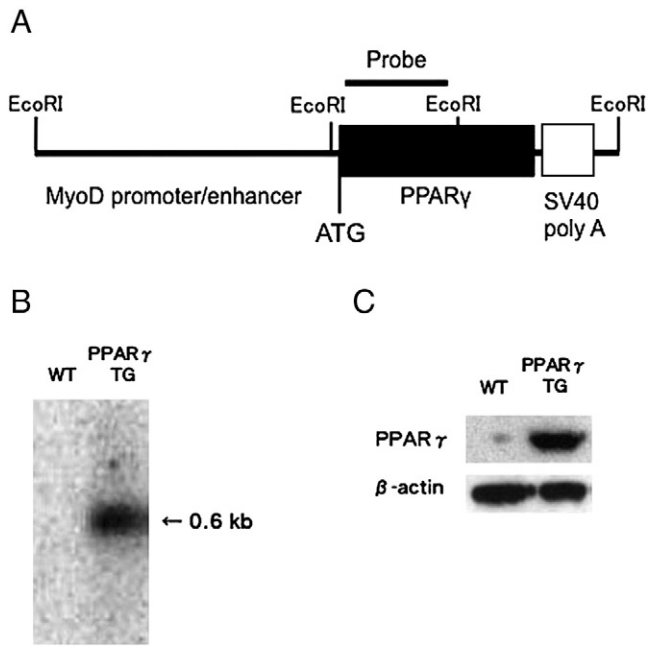

Fig. 1. Generation of transgenic mice. (A) Schematic of the transgene. Porcine PPARγ cDNA was regulated by MyoD promoter/enhancer. (B) Southern blot analysis of transgenic mice. Tail genomic DNA from wild-type mice (WT) and PPARγ transgenic mice (PPARγ TG) was digested with EcoRI and separated on an agarose gel. After digestion, the transgene generated a 0.6 kb PPARγ fragment. The individual blots were hybridized with a radiolabelled porcine PPARγ cDNA probe. (C) Western blot analysis of transgenic mice. Total protein from skeletal muscle was separated, blotted and hybridized with a PPARγ antibody. The β-actin was an indicator of equivalent protein loading. The detection of the transgene and the protein was performed in three individual mice.

2.3. Biochemical assays

A blood sample was obtained from the tail at the end of the dietary treatment. Blood plasma was used to colorimetrically measure glucose (K606-100, kits from BioVision, Mountain View, CA, USA), triacyglycerol (K622-100, BioVision) and free fatty acids (K612-100, BioVision). Plasma adiponectin was measured using a mouse adiponectin ELISA kit (B-Bridge International, Mountain View, CA, USA).

2.4. Histochemistry

Tissues were fixed in 10% formalin and embedded in paraffin. Sections (4 μm thick) were cut and stained with hematoxylin/eosin staining. For Oil red O staining, tissues were embedded in optimal cutting temperature (−20°C) and cryosectioned following a standard protocol with modifications by [16]. Frozen sections were subjected to standard Oil Red O staining [17].

2.5. Quantitative reverse transcription-PCR

Tissue RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed with a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Quantitative reverse transcriptase-PCR was performed using LightCycler 480 Instrument II (Roche Diagnostics, Indianapolis, IN, USA) and RealQ-PCR Master Mix Kit (Ampliqon, Herlev, Denmark). PCR was performed by 40 cycles of 95°C for 30 s, 58–60°C for 60 s, and 72°C for 30 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression was determined as the internal control gene. The sequence of primers for quantitative reverse transcription-PCR was listed in Table 1. The mRNA expression of each gene was normalized to its GAPDH mRNA expression in the same sample. Threshold cycle (C_t) values were obtained and relative gene expression was calculated using the formula $(1/2)^{Ct\ target\ genes - Ct\ GAPDH}$ [18].

2.6. Statistical analysis

The treatment effects were analyzed using an analysis of variance procedure to determine the main effects of PPARγ and diets. Statistical analysis of results was performed by a 2×4 factorial design (mouse genetic backgrounds and diet treatments) and Duncan's new multiple range test was used to evaluate differences among means (SAS Institute, Cary, NC, USA). A significant difference indicates that the P value is not greater than .05.

Table 1
Sequences for quantitative reverse transcription-PCR

Genes[a]	Primers	Annealed Temperature, °C
LPL	S 5′-AGGACCCCTGAAGACAC-3′	58
(BC003305)	A 5′-GGCACCCAACTCTCATA -3′	
GLUT-4	S 5′-CTTCTTTGAGATTGGCCCTGG-3′	58
(BC014282)	A 5′-AGGTGAAGATGAAGAAGCCAAGC-3′	
ADN	S 5′-GATGGCAGAGATGGCACTCC-3′	58
(NM 009605)	A 5′-CTTGCCAGTGCTGCCGTCAT-3′	
FAT	S 5′-GATGTGGAACCCATAACTGGATTCAC-3′	58
(BC010262)	A 5′-GGTCCCAGTCTCATTTAGCCACAGTA-3′	
SREBP-1c	S 5′-GGACCACGGAGCCATGG-3′	58
(NM 011480)	A 5′-GGAAGTCACTGTCTTGGTTGTTGA-3′	
FAS	S 5′-GCTGCGGAAACTTCAGGAAAT-3′	58
(BC046513)	A 5′-AGAGACGTGTCACTCCTGGACTT-3′	
ACO	S 5′-GCCCAACTGTGACTTCCATT-3′	58
(BC054727)	A 5′-GGCATGTAACCCGTAGCACT-3′	
CPT-1	S 5′-GCACTGCAGCTCGCACATTACAA-3′	60
(BC046383)	A 5′-CTCAGACAGTACCTCCTTCAGGAAA-3′	
MCAD	S 5′-GATCGCAATGGGTGCTTTTGATAGAA-3′	58
(BC013498)	A 5′-AGCTGATTGGCAATGTCTCCAGCAAA-3′	
LCAD	S 5′-GTAGCTTATGAATGTGTGCAACTC-3′	58
(BC027412)	A 5′-GTCTTGCGATCAGCTCTTTCATTA-3′	
ACC	S 5′-TGACAGACTGATCGAGAGAAAG-3′	60
(AY451393)	A 5′-TGGAGAGCCCCACACACA-3′	
GAPDH	S 5′-CAAGGTCATCCATGACAACTTTG-3′	60
(NM 007393)	A 5′-GGCCATCCACAGTCTTCTGG-3′	

S, sense; A, antisense; ADN, adiponectin.
[a] GenBank accession number is indicated parenthetically.

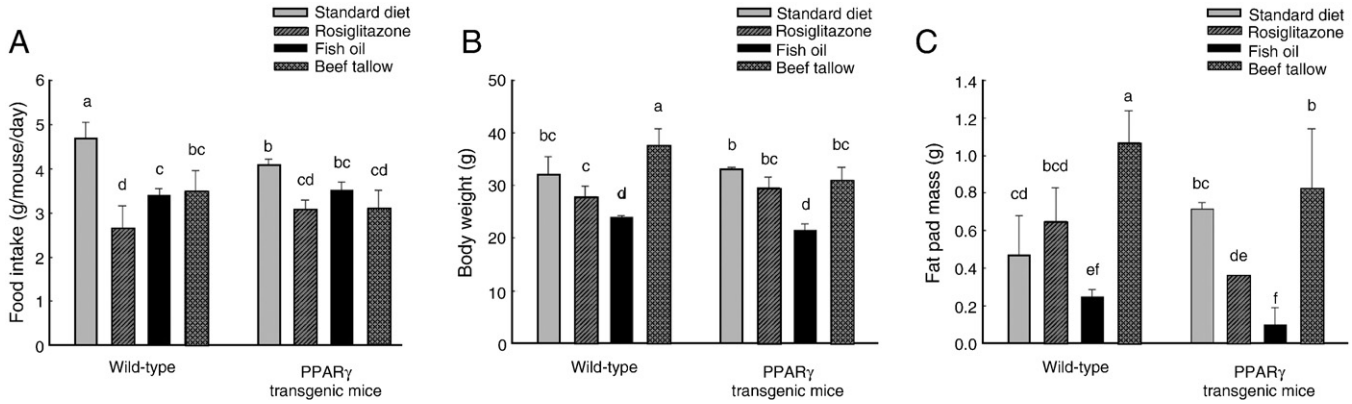

Fig. 2. Physiologic parameters in mice fed different diets. (A) Food intake was measured monthly in individually housed wild-type mice (WT) and myoblast determination protein-1 promoter/enhancer-porcine peroxisome proliferator-activated receptor γ transgenic mice (PPARγ transgenic mice). Values from each group are the means±S.E. (n=6). (B) Body weights of WT and PPARγ transgenic mice. Values from each group are the means±S.E. (n=3–11). (C) Weight of white fat pads of WT and PPARγ transgenic mice. Values from each group are the means±S.E. (n=3–11). [a–f]Means without a common letter differ, P≤.05.

3. Results

3.1. Expression of porcine PPARγ in transgenic mice

The constructs used to generate transgenic mice are shown in Fig. 1A. The transgene containing porcine PPARγ full length cDNA was driven by the mouse MyoD promoter and enhancer for muscle-specific expression. Tail genomic DNA from transgenic founder mice was digested by EcoRI for detection of porcine PPARγ. The Southern blot detected porcine PPARγ using isotope-labeled porcine PPARγ cDNA fragments, which completely hybridize to porcine PPARγ [11]. Successful integration of the transgene was confirmed by the Southern blot (Fig. 1B). Total protein from skeletal muscle was used to detect PPARγ by Western blot. In wild-type mice, endogenous PPARγ expression in skeletal muscle was weak (Fig. 1C). In contrast, in transgenic mice with porcine PPARγ integration, PPARγ was highly expressed in skeletal muscle (Fig. 1C).

3.2. The effect of diet on phenotype of transgenic mice

Dietary treatments had a significant effect on feed intake (P<.01), body weight (P<.01) and fat pad weight (P<.01, Fig. 2).

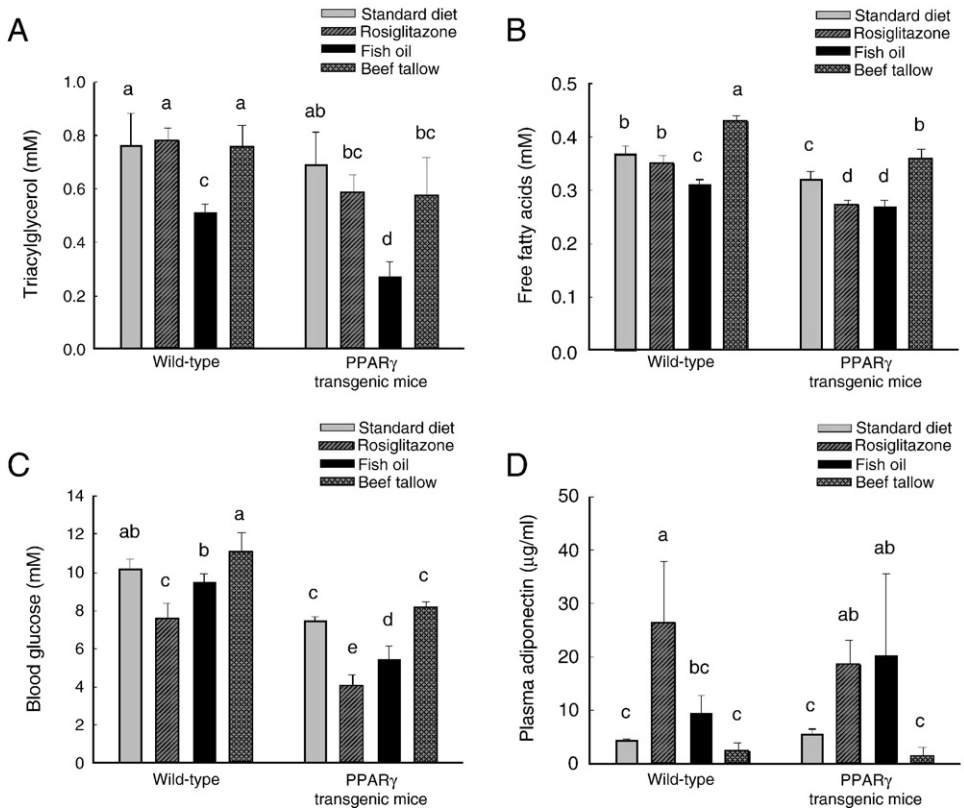

Fig. 3. Plasma metabolites in mice fed different diets. (A) Plasma triacylglycerol levels of wild-type mice (WT) and myoblast determination protein-1 promoter/enhancer-porcine peroxisome proliferator-activated receptor γ transgenic mice (PPARγ transgenic mice). (B) Plasma free fatty acid levels of WT and PPARγ transgenic mice. (C) Blood glucose levels of WT and PPARγ transgenic mice. (D) Plasma adiponectin concentrations of WT and PPARγ transgenic mice. Values from each group are the means±S.E. (n=3). [a–e]Means without a common letter differ, P≤.05.

The mouse genetic backgrounds (wild-type versus transgenic) had no effect on feed intake, body weight or fat pad weight ($P>.05$). Compared with standard diet-fed wild-type mice, PPARγ ligand-fed (rosiglitazone) mice had a significantly lower feed intake (Fig. 2A, $P<.05$), but dietary rosiglitazone had no significant effect on body weight (Fig. 2B) or fat pad weight (Fig. 2C). PPARγ transgenic mice ate less than wild-type mice when fed with the standard diet. Fish oil feeding significantly decreased feed intake and body and fat pad weight in wild-type mice. In PPARγ-transgenic mice, fish oil feeding did not significantly decrease feed intake, but significantly decreased body and fat pad weight. In wild-type mice, dietary beef tallow decreased feed intake but increased body weight. The beef tallow fed mice had greater fat pad weight than the fish oil-fed mice.

Both diet treatments and genetic backgrounds had significant effects on plasma triacylglycerol, plasma free fatty acid and plasma glucose. Fish oil feeding reduced plasma triacylglycerol in both the wild-type and transgenic mice (Fig. 3A). Compared with wild-type mice, PPARγ transgenic mice had an overall low plasma free fatty acid concentration across all diets (Fig. 3B). On each diet, PPARγ transgenic mice had a lower plasma glucose concentration than wild-type mice (Fig. 3C). Feeding wild-type mice a PPARγ-ligand, rosiglitazone markedly reduced plasma glucose concentration and the effect was enhanced in PPARγ transgenic mice (Fig. 3C). Dietary rosiglitazone increased the plasma adiponectin concentration in both genotypes (Fig. 3D). Fish oil feeding numerically increased plasma adiponectin concentration in wild-type mice and increased adiponectin in PPARγ transgenic mice (Fig. 3D).

Adipocyte size was not different between wild-type and transgenic mice when fed either the standard or the rosiglitazone diets (Fig. 4). Fish oil feeding markedly decreased adipocyte size in wild-type mice and adipocyte size was further decreased in the PPARγ transgenic mice fed fish oil. Compared to the standard diet, adipocyte size was increased in wild-type mice fed beef tallow. However, in PPARγ transgenic mice, adipocyte size was the same when mice were fed either the standard or beef tallow diets.

Hepatocyte lipid droplet accumulation was not observed in wild-type mice fed either the standard or rosiglitazone diet (Fig. 5A). Hepatocytes from PPARγ transgenic mice had lipid droplet accumulation when fed rosiglitazone compared to the standard diet (Fig. 5B). Feeding wild-type or PPARγ transgenic mice fish oil slightly increased hepatic lipid droplet accumulation. On the other hand, a severe fatty liver was observed in wild-type mice fed the beef tallow diet and to a lesser extend in the PPARγ transgenic mice fed the beef tallow diet (Fig. 5B). These results indicate that high-fat feeding caused fatty liver symptoms, but the symptoms in fish oil fed mice were of lower

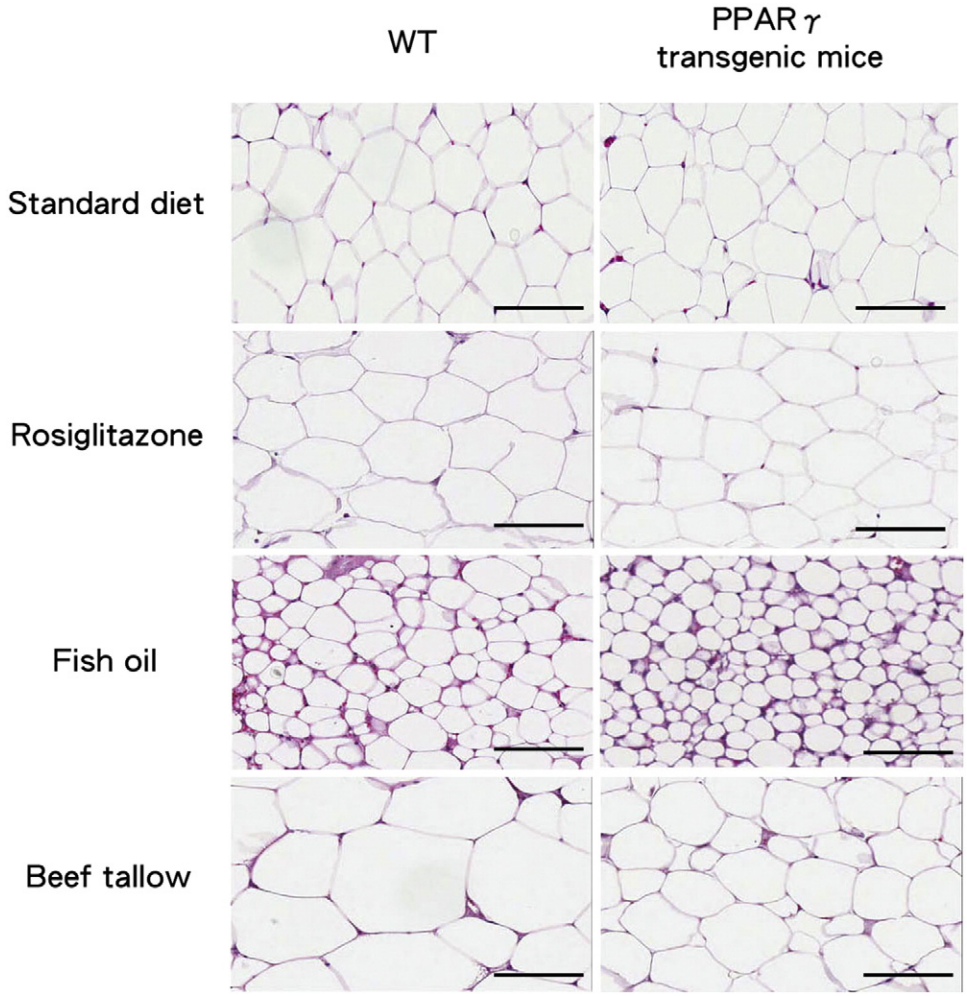

Fig. 4. Histology of adipose tissue sections from wild-type mice and transgenic mice. Adipose tissue from reproductive fat pads was sectioned and stained with hematoxylin/eosin. Histology of adipose tissue from wild-type mice (WT) and myoblast determination protein-1 promoter/enhancer-porcine peroxisome proliferator-activated receptor γ transgenic mice (PPARγ transgenic mice) when fed standard diet, rosiglitazone, fish oil or beef tallow (original magnification ×200). Bars indicate a length of 100 μm. Three mice were carried out, and one representative result was shown on the figure.

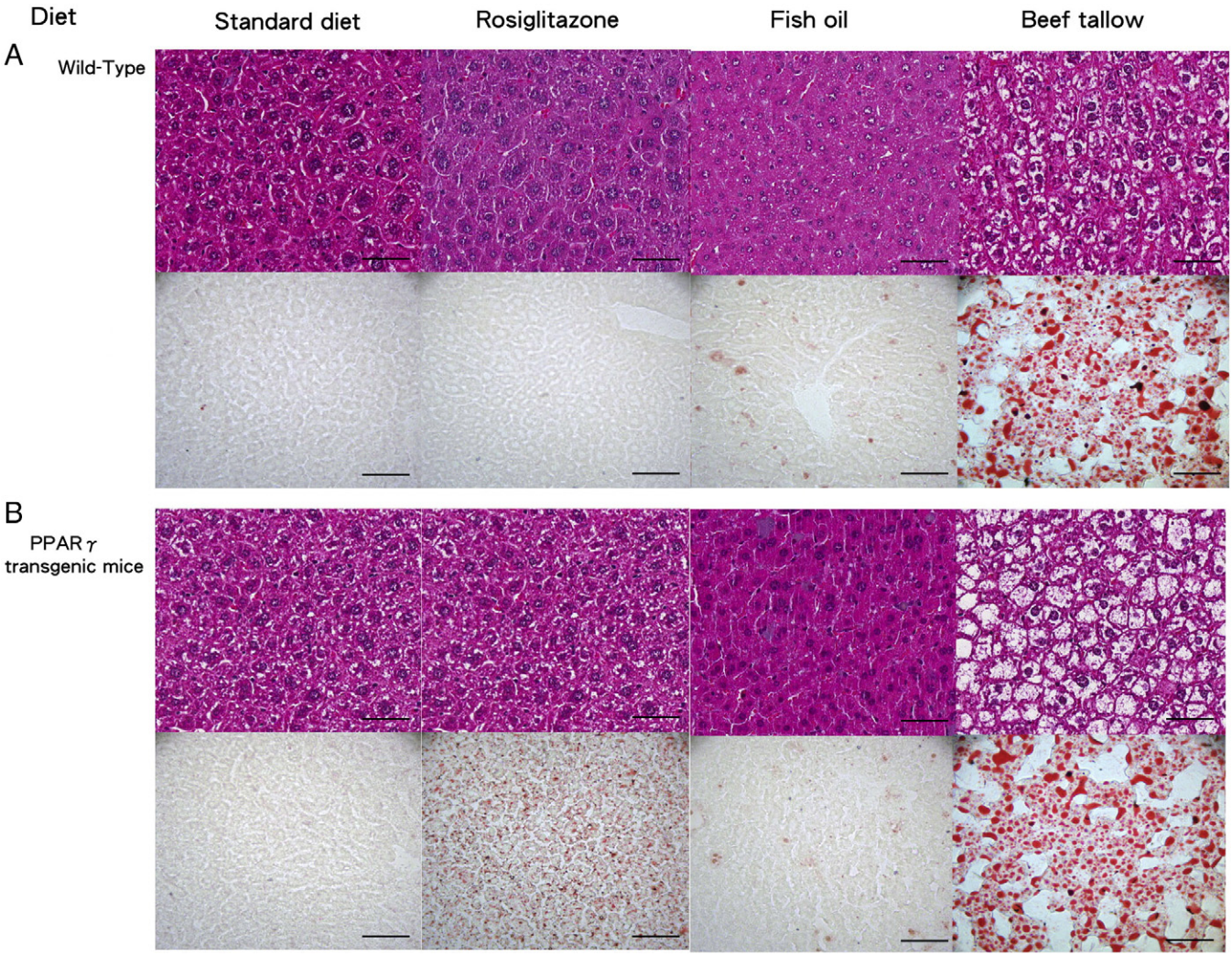

Fig. 5. Histology and Oil red O staining of the liver sections from wild-type mice and transgenic mice. Liver was cryosectioned and stained with Oil red O. (A) Hematoxylin/eosin staining and Oil red O staining of the liver from wild-type mice (WT) and myoblast determination protein-1 promoter/enhancer-porcine peroxisome proliferator-activated receptor γ transgenic mice (PPARγ transgenic mice) when fed standard diet and rosiglitazone. (B) Hematoxylin/eosin staining and Oil red O staining of the liver from WT and PPARγ transgenic mice when fish oil and beef tallow. (original magnification ×200). Bars indicate a length of 100 μm. Three mice were carried out and one representative result was shown on the figure.

magnitude than in mice fed beef tallow. Polyunsaturated fatty acids were able to diminish lipid deposition in liver.

3.3. Muscle-specific PPARγ overexpression has an effect on adipogenic genes and glucose uptake genes in the skeletal muscle

The mRNA level of the PPARγ-regulated adipogenic marker gene, lipoprotein lipase (LPL), was low in wild-type mice even in the presence of the PPARγ ligand, rosiglitazone, fish oil or beef tallow (Fig. 6A). PPARγ transgenic mice had a high level of LPL mRNA when fed rosiglitazone. These results demonstrated that porcine PPARγ is functional in transgenic mice and that an exogenous ligand greatly enhanced LPL expression in both genotypes. Fish oil greatly enhanced the expression of LPL in PPARγ transgenic mice, whereas dietary beef tallow did not change the expression of LPL in either genotype. The mRNA level for the fatty acid uptake gene, fatty acid translocase (FAT) was increased in wild-type mice when fed fish oil (Fig. 6B). In PPARγ transgenic mice, FAT mRNA levels were increased by dietary beef tallow, increased to a greater extent by rosiglitazone and increased even more so by fish oil. The mRNA level of SREBP-1c, a nuclear transcription factor involved in lipogenesis, was unaltered in wild-type mice when fed any of the diets (Fig. 6C). In PPARγ transgenic mice, the SREBP-1c mRNA levels were elevated by rosiglitazone feeding. The mRNA levels for the SREBP-1c target gene, fatty acid synthase (FAS), were not different in wild type mice fed any of the diets (Fig. 6D). Feeding PPARγ transgenic mice rosiglitazone or beef tallow increased FAS mRNA levels. In contrast, the mRNA level for FAS was decreased when fish oil was provided.

The mRNA for the glucose uptake gene, glucose transporter-4 (GLUT-4) was increased in wild-type mice and even more so in PPARγ transgenic mice fed rosiglitazone compared to the standard diet (Fig. 6E). High-fat feeding did not change the GLUT-4 mRNA levels in wild-type mice. In PPARγ transgenic mice, dietary fish oil, but not beef tallow, caused the GLUT-4 mRNA levels to increase. The pattern for expression of mRNA for another glucose metabolism-related and PPARγ-regulated gene, adiponectin (ADN) was similar to that for GLUT-4 in both wild-type and PPARγ transgenic mice (Fig. 6F). The interaction of the two major factors (mouse genetic backgrounds and diet treatments) on the expression of LPL, FAT, and GLUT4 mRNA was significant ($P < 0.05$), indicating that the regulation of these genes by PPARγ transgene depended on the dietary treatments.

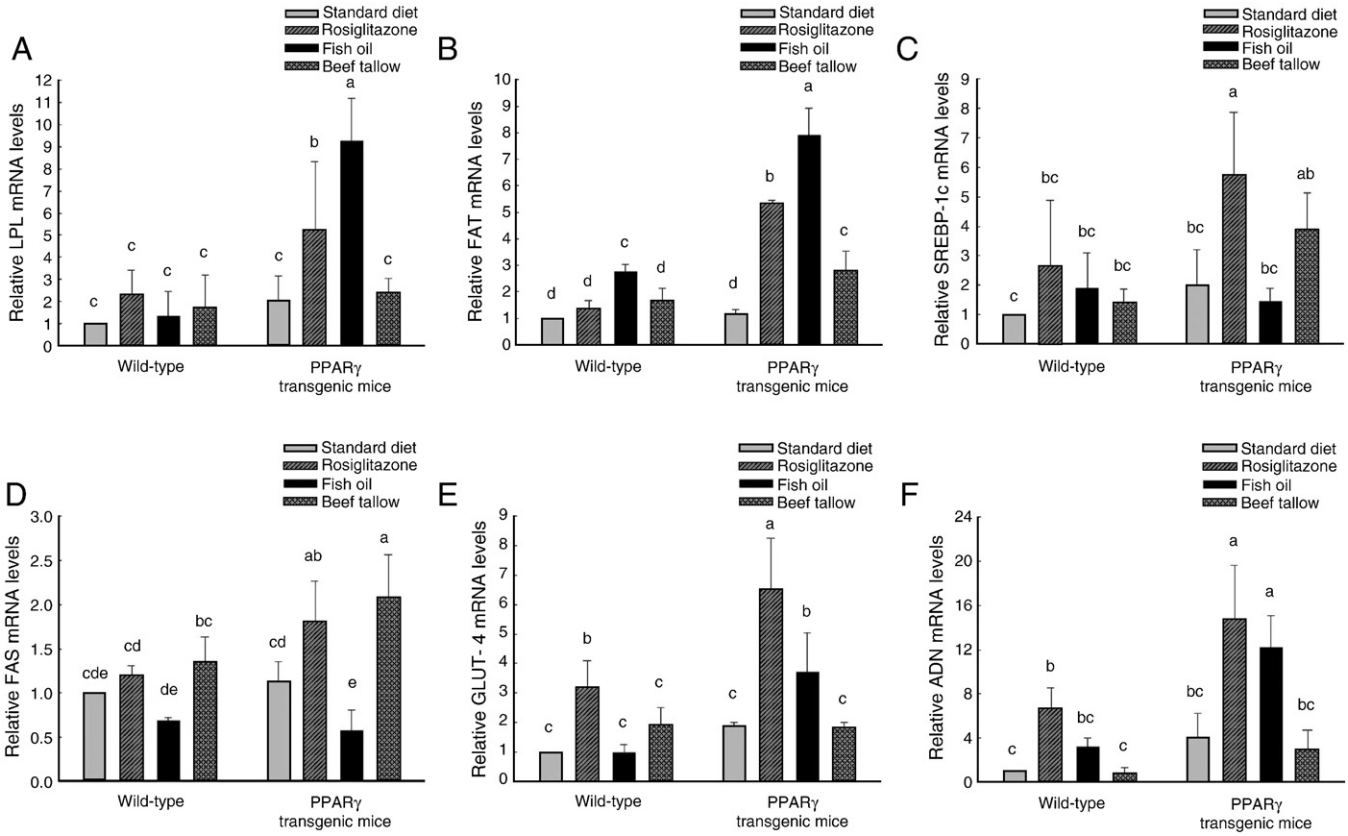

Fig. 6. Expression of adipogenic and lipogenic genes in muscle. Total muscle RNA from wild-type mice (wild-type) and myoblast determination protein-1 promoter/enhancer-porcine peroxisome proliferator-activated receptor γ transgenic mice (PPARγ transgenic mice) was isolated at the end of the 4-month dietary treatments. The mRNA levels were measured by quantitative reverse transcription-PCR. The expression of adipogenic and lipogenic marker genes (LPL, GLUT-4, ADN, FAT, SREBP-1c and FAS) were determined and normalized to the mRNA for GAPDH. Values from each group are the means±S.E. (n=3–6). [a–e]Means without a common letter differ, $P \leq .05$.

3.4. Hepatic lipid metabolism gene expression

We examined mRNA levels involved in fatty acid oxidation and lipogenesis in liver. Fish oil-fed wild-type and PPARγ transgenic mice had increased acyl-CoA oxidase (ACO) mRNA levels compared to mice fed the other diets (Fig. 7A). The patterns for mRNA levels of mitochondrial fatty acid oxidation genes including carnitine palmitoyltransferase 1 (CPT-1), medium chain acyl-CoA dehydrogenase (MCAD) and long-chain acyl-CoA dehydrogenase (LCAD) were complex. Fish oil feeding increased MCAD expression in wild-type and PPARγ transgenic mice, but decreased LCAD expression in transgenic mice (Fig. 7C and D). Beef tallow feeding increased expression of CPT1 and MCAD in transgenic mice, as well as MCAD and LCAD in wild-type mice (Fig. 7B–D). Rosiglitazone in the diet increased the SREBP-1c mRNA levels in PPARγ transgenic mice (Fig. 7E). The mRNA level of SREBP-1c was not changed in either genotype when mice were fed fish oil compared to the standard diet (Fig. 7E). For the lipogenic enzyme, acetyl-CoA carboxylase (ACC), rosiglitazone feeding elevated the mRNA levels in PPARγ transgenic mice, but not in wild-type mice (Fig. 7F). The ACC mRNA level was increased in transgenic mice when fed fish oil, but not when fed beef tallow (Fig. 7F). Dietary treatments and genetic backgrounds interacted to affect CPT-1, LCAD, and ACC mRNA expression (P<.05), indicating that the effect of PPARγ transgene on the expression of these genes depended on the dietary treatments.

4. Discussion

Adipocyte differentiation is regulated by several transcription factors. These factors promote cell morphologic conversion, lipogenic gene expression and triacylglycerol accumulation. PPARγ, a ligand-activated nuclear transcription factor, has been clearly demonstrated to be an important key factor in adipogenesis [1]. In addition to adipocyte differentiation, PPARγ also participates in regulation of insulin sensitivity [19]. Skeletal muscle is the largest organ involved in regulation of glucose homeostasis. Deletion of PPARγ in skeletal muscles causes severe insulin resistance [2,20], confirming that muscle PPARγ has an important role in regulation of whole-body insulin sensitivity.

To activate PPARγ, a ligand is needed to bind to its ligand binding domain. The antidiabetic drugs, thiazolidinediones (TZD) are potent and effective ligands for PPARγ [21]. The TZD promote insulin-stimulated glucose disposal in skeletal muscle and are widely used to treat Type 2 diabetes [22]. Fish oil, rich in EPA and DHA, has similar effects on insulin sensitization. Dietary fish oil prevents insulin resistance in high-fat-fed rats [23] by regulating adiponectin secretion [9]. Studies in vitro suggest that DHA has high affinity for human [5], monkey [7] and porcine PPARγ [12]. In the current study, we found that plasma glucose concentration was not affected by fish oil feeding in wild-type mice but was significantly decreased in fish oil-fed transgenic mice expressing high levels of PPARγ in skeletal muscle. Plasma adiponectin was increased in wild-type and PPARγ transgenic mice by oral rosiglitazone, the PPARγ ligand. Plasma adiponectin was numerically increased in wild-type and statistically increased in PPARγ transgenic mice by dietary fish oil. The adipose tissue adiponectin mRNA pattern duplicated the plasma concentrations. Perhaps the minimal amount of PPARγ in wild-type mouse skeletal muscle was sufficient to cause the change in adiponectin. In PPARγ transgenic mice, with considerable skeletal muscle PPARγ (Fig. 1B,C). Thus, the increased skeletal muscle GLUT-4 and adiponectin gene

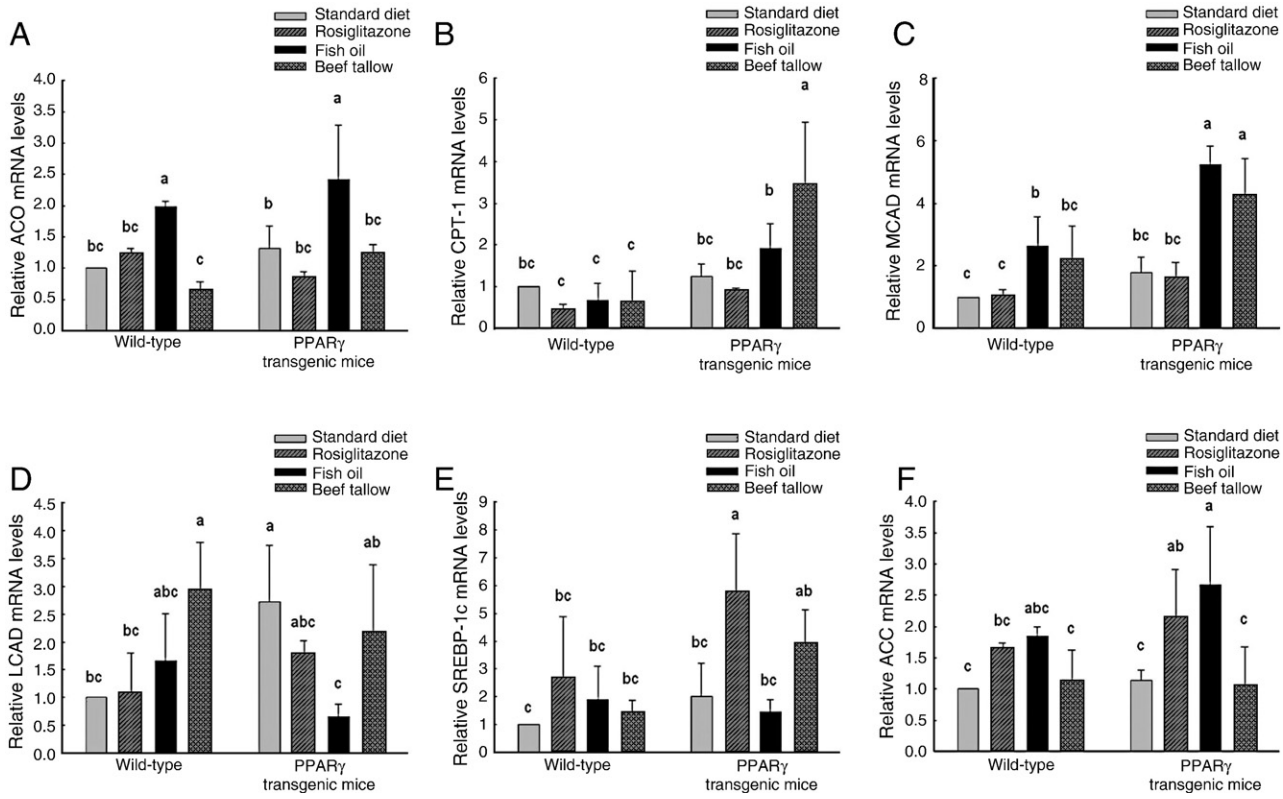

Fig. 7. Expression of β-oxidation genes and lipogenic genes in liver. Total liver RNA from wild-type mice (wild-type) and myoblast determination protein-1 promoter/enhancer-porcine peroxisome proliferator-activated receptor γ transgenic mice (PPARγ transgenic mice) was isolated at the end of the 4-month dietary treatments. The mRNA levels were measured by quantitative reverse transcription-PCR. The expression of β-oxidation genes (ACO, CPT-1, LCAD, MCAD) and lipogenic genes (SREBP-1c and ACC) were determined and normalized to the mRNA for GAPDH. Values from each group are the means±S.E. (n=3–6). $^{a-f}$Means without a common letter differ, $P \leq .05$.

expression induced by rosiglitazone feeding in both genotypes and by fish oil feeding in PPARγ transgenic mice appeared to modify insulin sensitivity to lower plasma glucose. Our data demonstrated that The PPARγ-regulated glucose metabolism genes, GLUT-4 and ADN were dramatically increased in skeletal muscle of PPARγ transgenic mice when fed rosiglitazone or fish oil, suggesting activation of the overexpressed PPARγ transgene by either ligand.

Addition of a PPARγ-ligand, a thiazolidinedione or a selected fatty acid to the medium induces transdifferentiation of myocytes into adipocytes [24]. Ectopic expression of PPARγ triggers adipogenesis in myoblasts [11,25]. However, there are few evidences for myoblast trasnsdifferentiation into adipocytes in vivo. Recently, transient introduction of PPARγ into rat skeletal muscle by electroporation indicated that ectopic PPARγ was not able to induce adipogenesis in skeletal muscle [26]. In the current studies, transgenic mice permanently expressed porcine PPARγ in skeletal muscle. Wild-type and PPARγ transgenic mice were fed a PPARγ ligand, rosiglitazone or fish oil for four months. The adipogenic genes (LPL, FAT, SREBP-1c and FAS) were markedly up-regulated by rosiglitazone feeding. In PPARγ transgenic mice, fish oil feeding increased LPL and FAT, but not SREBP-1c or FAS; however, Oil Red O stained muscle sections indicate no lipid accumulation in skeletal muscle (data not shown). In our previous studies in vitro, adipogenic genes were activated by rosiglitazone when myoblasts expressing porcine PPARγ were cultured in myogenic medium (the proper environment for myotube development with no added insulin). It should be noted that the number of modified cells containing lipid-droplets was rare and the size of the lipid droplets was very small [11]. Thus, although adipogenic genes are increased in transgenic skeletal muscle expressing high levels of PPARγ and exposed to PPARγ ligands, additional hormones/growth factors (e.g., insulin, dexamethasone, etc.) probably are needed.

In conclusion, we demonstrated that adipogenic genes and glucose metabolism genes were elevated in PPARγ transgenic mice when fed fish oil. This transgenic mouse model provided direct evidence to demonstrate PUFA, especially EPA and DHA, regulate glucose homeostasis through interaction with PPARγ.

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