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Transgenic expression of mutant peroxisome proliferator–activated receptor γ in liver precipitates fasting–induced steatosis but protects against high-fat diet–induced steatosis in mice

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Abstract

Steatosis is one of the most common liver diseases and is associated with the metabolic syndrome. A line of evidence suggests that peroxisome proliferator–activated receptor (PPAR) α and PPARγ are involved in its pathogenesis. Hepatic overexpression of PPARγ1 in mice provokes steatosis, whereas liver-specific PPARγ disruption ameliorates steatosis in *ob/ob* mice, suggesting that hepatic PPARγ functions as an aggravator of steatosis. In contrast, PPARα-null mice are susceptible to steatosis because of reduced hepatic fatty acid oxidation. PPARγ with mutations in its C-terminal ligand-binding domain (L468A/E471A mutant PPARγ1) have been reported as a constitutive repressor of both PPARα and PPARγ activities in vitro. To elucidate the effect of cosuppression of PPARα and PPARγ on steatosis, we generated mutant PPARγ transgenic mice (Liver mt PPARγ Tg) under the control of liver-specific human serum amyloid P component promoter. In the liver of transgenic mice, PPARα and PPARγ agonist–induced augmentation of the expression of downstream target genes of PPARα and PPARγ, respectively, was significantly attenuated, suggesting PPARα and PPARγ cosuppression in vivo. Suppression of PPARα and PPARγ target genes was also observed in the fasted and high-fat–fed conditions. Liver mt PPARγ Tg were susceptible to fasting-induced steatosis while being protected against high-fat diet–induced steatosis. The opposite hepatic outcomes in Liver mt PPARγ Tg as a result of fasting and high-fat feeding may indicate distinct roles of PPARα and PPARγ in 2 different types of nutritionally provoked steatosis.

1. Introduction

Nonalcoholic fatty liver disease is now recognized as 1 of the common features of the metabolic syndrome [1-4], a cluster of visceral fat obesity, insulin resistance, dyslipidemia, and hypertension [5-7] and is a major contributor to obesity-related morbidity and mortality [1,2]. Intrahepatic lipid accumulation is responsible for systemic as well as hepatic insulin resistance [8,9].

The mechanisms underlying liver steatosis are complex and multifactorial [1,2,4,10,11]. However, a line of evidence suggests that peroxisome proliferator–activated receptor (PPAR) α and PPARγ, which regulate lipid metabolism [12], are involved in its pathogenesis [13-16]. Synthetic agonists for PPARα [17] and γ [18] have been widely used as lipid-lowering and antidiabetic agents, respectively, thus providing a therapeutic possibility for the treatment of steatosis by PPARα and/or PPARγ modulation [19,20]. PPARα is highly expressed in the liver and regulates a set of enzymes crucial for fatty acid oxidation [21,22]. PPARα-null mice exhibit fatty liver in elderly male animals [23], which is aggravated by either a high-fat diet (HFD) or

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starvation [14,24]. PPARγ is a key regulator of adipocyte differentiation and lipid storage [25,26]. Recent studies have revealed that PPARγ expression in the liver is augmented in murine steatosis [27-30]. Adenovirus-mediated overexpression of PPARγ in the liver provokes steatosis [31], whereas liver-specific disruption of PPARγ substantially ameliorates severe steatosis in *ob/ob* mice [15] and A-ZIP/F-1 mice, a murine model of lipoatrophic diabetes [16].

Mutations in the C-terminal (AF-2) domain of some nuclear receptors are known to generate proteins with a strong dominant negativity [32]. For example, L468A/E471A double-mutant of PPARγ1 has been reported to serve as a dominant-negative on PPARα and PPARγ1/PPARγ2 by recruiting and binding transcriptional corepressors, including the silencing mediator of retinoid and thyroid receptors and the nuclear corepressor [32].

To unravel the impact of L468A/E471A mutant PPARγ1 expression in the liver, we generated mutant PPARγ transgenic mice (Liver mt PPARγ Tg), in which the transgene (L468A/E471A mutant PPARγ1 cDNA) is exclusively expressed in the liver. The Liver mt PPARγ Tg were subjected to 2 distinct steatogenic stimuli (fasting and high-fat feeding) to examine the effect of mutant PPARγ on steatosis. In Liver mt PPARγ Tg, fasting-induced steatosis is aggravated, whereas HFD-induced steatosis is ameliorated, indicating that mutant PPARγ has distinct impacts on the pathophysiology of fasting- and HFD-induced steatosis.

2. Materials and methods

2.1. Plasmid construction, transfection, and luciferase assay

Full-length mouse PPARγ1 cDNA coding sequence was isolated from mouse epididymal adipose tissue total RNA by reverse transcriptase–polymerase chain reaction using Superscript II reverse transcriptase (Invitrogen, Carlsbad, Calif) and Takara ExTaq (Takara, Otsu, Japan). Three base-pair substitutions were introduced by site-directed mutagenesis that resulted in 2 missense mutations in the C-terminal (AF-2) domain of mouse PPARγ1 [32]. The resultant cDNA encoding L468A/E471A double-mutant PPARγ1 was cloned into the pCMX eukaryotic expression vector (a gift from Dr Kazuhiko Umesono, Nara Institute of Science and Technology, and Dr Ronald M. Evans, Salk Institute for Biological Studies) [33] to give pCMX-L468A/E471A PPARγ1 construct. Wild-type murine PPARα and PPARγ1 cDNA were also cloned into the pCMX vector and cotransfected with pCMX-L468A/E471A PPARγ1 and PPAR-responsive PPREx3-tk-LUC reporter [34] into human embryonic kidney (HEK) 293 cells using LipofectAMINE plus transfection kit (Invitrogen) [35] according to manufacturer's instructions. Cells were cultured for 36 hours in Dulbecco modified Eagle medium containing 10% bovine serum albumin [36]. Luciferase activity was measured by dual luminescence luciferase kit (Promega, Madison, Wis) as previously reported [36].

2.2. Animals

A fusion gene comprising the human serum amyloid P component promoter and mouse L468A/E471A PPARγ1 cDNA was designed so that the mutant PPARγ1 expression might be targeted to the liver [37,38]. The purified *Hind*III-*Xho*I fragment was microinjected into the pronucleus of fertilized C57BL/6N (Charles River Japan, Co, Tokyo, Japan) mouse eggs, and transgenic founder mice were generated [38]. The transgenic mice were identified by Southern blot analysis of the tail genomic DNA using cDNA fragment as a probe [38]. F2-3 heterozygous male mice were used for further experiments. Animals were individually housed and maintained on a chow diet (CE-1, 3.42 kcal/g [14.31 kJ/g], 11.6 kcal% [11.6 kJ%] fat, Japan CLEA, Tokyo, Japan) and on a 12-hour light cycle (light, 9:00 AM to 9:00 PM). All animals had ad libitum access to food and water unless otherwise mentioned. For drug administration, mice were fed power diet (CRF-1, Oriental Yeast Co, Ltd, Tokyo, Japan) containing 0.01% (wt/wt) pioglitazone (Takeda Chemical Industries, Osaka, Japan) for 3 weeks or 0.5% (wt/wt) bezafibrate (Kissei Pharmaceutical Co, Ltd, Nagano, Japan) for 6 days. Control mice were fed powder diet without drugs. The average daily food intake of powder diet–fed animals was 0.125 ± 0.006 g/g of body weight (n = 5) (no difference among mice fed control diet and diet containing pioglitazone or bezafibrate [n = 5]), which corresponds to ~12.5 mg/kg per day for pioglitazone and ~625 mg/kg per day for bezafibrate. For fasting experiment, 10-week-old mice were deprived of chow diet at 9:00 AM and were fasted for 72 hours, after which they were killed for the study. HFD (D12493, 5.24 kcal/g [21.92 kJ/g], 60 kcal% [60 kJ%] fat, Research Diets, New Brunswick, NJ) was initiated from 10 weeks of age and was continued for 19 weeks. Animals were anesthetized with diethyl ether before sampling. All animal experiments were performed according to National Institutes of Health guidelines and were approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University.

2.3. Northern blot analysis and RNase protection assay

Liver total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, Calif) [38] and digested with RNase-free DNase (Qiagen, Hilden, Germany) according to manufacturer's instructions. Northern blot analysis was performed as previously described [38] using liver total RNA (40 μg per lane). RNase protection assay kit (Ambion, Austin, Tex) was used to hybridize liver and adipose tissue total RNA with radiolabeled RNA probe [39] generated from wild-type cDNA sequences spanning mutated nucleotides in L468A/E471A PPARγ1. To confirm the presence of transgene-derived mRNA, double-stranded RNA was digested with RNase A/T_1, electrophoresed on urea polyacrylamide gel, and subjected to autoradiography as previously described [39].

2.4. Liver Histology

Liver samples were fixed in 10% formalin neutral-buffered solution (Wako, Osaka, Japan) and embedded in paraffin wax, and 5-μm sections were cut and mounted on glass slides. After dehydration, the sections were stained with hematoxylin-eosin (H-E) [38] for histological examination.

2.5. Plasma assays and liver triglyceride content measurement

Plasma levels of alanine aminotransferase activity (Sigma, St Louis, Mo), triglyceride (Triglyceride-E test, Wako), non-esterified fatty acids (NEFA-C test, Wako), and insulin (Insulin ELISA kit, Morinaga, Yokohama, Japan) concentrations were determined according to manufacturer's instructions. Liver was homogenized in isopropyl alcohol and heptane, lipids were extracted [40], and the triglyceride content was determined by a kit using calorimetry (Triglyceride-E test, Wako).

2.6. Statistical analyses

Data were expressed as the mean ± SEM unless otherwise mentioned. Comparison between genotypes or animal groups was assessed by Student *t* test or analysis of variance, followed by Fisher probable least significant difference test where applicable.

3. Results

3.1. mRNA levels for PPARα and PPARγ were both augmented in fasting- and HFD-induced steatosis

Ten-week-old C57BL/6N wild-type male mice were fed ad libitum or food-deprived for 72 hours (n = 5). Another group of 10-week-old wild-type male mice was maintained either on a chow diet or HFD for 19 weeks (n = 5). The animals were killed, and the hepatic mRNA levels for PPARα and PPARγ were measured by Northern blot analysis. Hepatic expression of both PPARα and PPARγ was augmented in steatosis provoked by either fasting or HFD (Fig. 1).

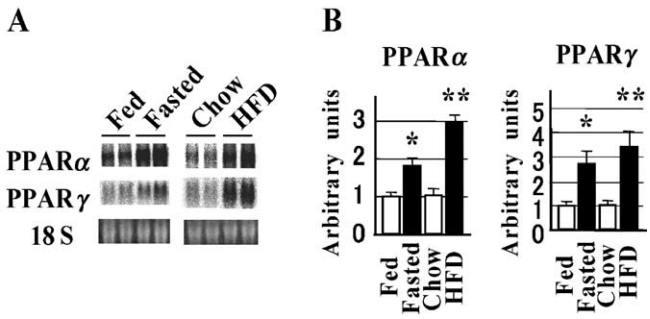

Fig. 1. Northern blot analysis of hepatic mRNA levels for PPARα and PPARγ in fasting-induced and high-fat diet–induced steatosis. Representative blots (A) and quantified data normalized by the intensity of ethidium bromide–stained 18S rRNA (B) (n = 5 for each group), *P < .05 vs Fed, **P < .05 vs Chow.

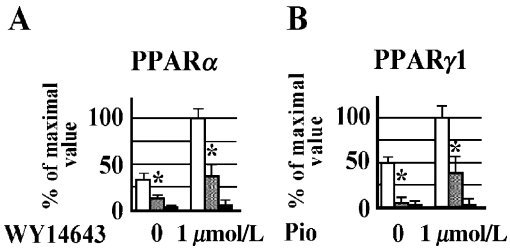

Fig. 2. PPRE-luciferase activity of PPARα (A) and PPARγ1 (B) (open bars) in the presence or absence of WY14643 (PPARα agonist) and Pio (pioglitazone: PPARγ agonist), respectively. Shaded bars represent the activity of PPARα (A) and PPARγ1 (B) with the cotransfection of L468A/E471A mutant PPARγγ1 cDNA. Dark bars represent the pCMX vector alone. Data are expressed as percent of maximal value (n = 6 for each bar), *P < .05 vs PPARα (A) or PPARγ1 (B).

3.2. Mutant PPARγ exhibits a dominant-negative effect on both PPARα and PPARγ in vitro

L468A/E471A double-mutant PPARγ1 has been reported to work in a dominant-negative fashion on PPARγ1 and γ2 in vitro [32]. Furthermore, authors of the report have shown that this mutant is capable of suppressing PPARα transcriptional activity [32]. We therefore hypothesized that the L468A/E471A mutant might be capable of suppressing the function of both PPARα and PPARγ. As expected, L468A/E471A mutant PPARγ1 repressed the transcriptional activities of PPARα and PPARγ1 when cotransfected into HEK293 cells (Fig. 2) (n = 6 for each column). A dominant-negative effect was observed in both the presence and absence of each specific agonist for PPARα and PPARγ (Fig. 2). We then used L468A/E471A mutant PPARγ1 to suppress PPARα and γ activities in the liver in vivo.

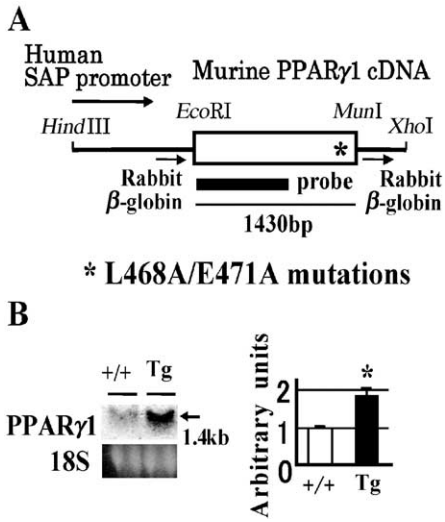

Fig. 3. DNA construct for the generation of Liver mt PPARγ Tg (A). The position of the L468A/E471A mutations in the construct is indicated by an asterisk. B, Northern blot analysis of hepatic mRNA level for PPARγ in wild-type mice (+/+) and Liver mt PPARγ Tg (Tg). The DNA probe used for Northern blot analysis is indicated in A. Representative blot and quantified data normalized by the 18S rRNA expression level are shown (n = 5 for each genotype), *P < .05 vs +/+.

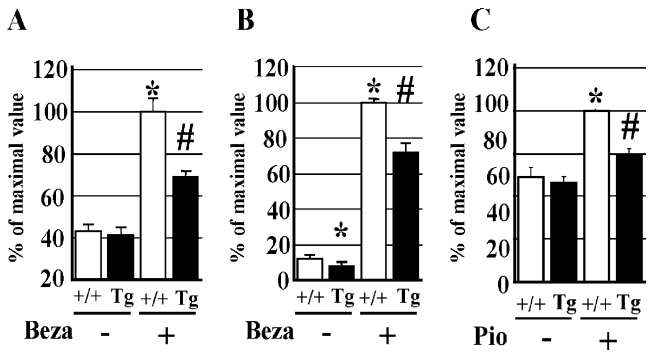

Fig. 4. Northern blot analysis of hepatic mRNA levels for PPARα and PPARγ target genes. The levels of mRNA expression for AOX (A) and CPT-I (B) in bezafibrate (Beza)–treated and untreated Liver mt PPARγ Tg (Tg) and nontransgenic littermates (+/+). The level of mRNA expression for ACC (C) in pioglitazone (Pio)–treated and untreated mice. Quantified data are normalized by the 18S rRNA expression level. Open bars, +/+; dark bars, Liver mt PPARγ Tg (n = 5 for each group), $*P < .05$ vs control +/+, $\#P < .05$ vs Beza +/+ (A and B) or Pio +/+ (C).

3.3. Generation of Liver mt PPARγ Tg

L468A/E471A mutant PPARγ1 was cloned into the downstream of human serum amyloid P component promoter so that transgene expression might be targeted specifically to the liver [37,38], and *Hind*III-*Xho*I fragment was used for microinjection (Fig. 3A). Liver expression of the transgene was confirmed by a 1.95 ± 0.30–fold increase in hepatic PPARγ1 expression by Northern blot analysis (Fig. 3B) (n = 5) and by the expression of transgene-specific mRNA in an RNase protection assay (data not shown) (n = 4). The liver-specific mutant PPARγ transgenic mice were named Liver mt PPARγ Tg.

To confirm the dominant-negative effect in vivo, we examined the mRNA levels for reported downstream target genes of PPARα and PPARγ. In the absence of a PPARα agonist, the liver mRNA level for acyl-CoA oxidase (AOX), a representative PPARα target gene in the liver [21], was not altered in the transgenic mice (Fig. 4A). However, when Liver mt PPARγ Tg were treated with a PPARα agonist, agonist-induced enhancement of AOX expression was significantly suppressed (Fig. 4A). Disturbed up-regulation of AOX by peroxisome proliferator in Liver mt PPARγ Tg is in line with data from PPARα-null mice, which exhibit unaltered basal AOX expression in the liver with an abolished response to PPARα agonist [21]. In contrast, carnitine palmitoyl transferase I (CPT-I), another PPARα target gene [14], was partially but significantly suppressed in Liver mt PPARγ Tg in both the presence and absence of PPARα agonist (Fig. 4B). Augmentation of the expression of acetyl-CoA carboxylase (ACC), a reported PPARγ target gene in the liver [15], in the presence of a PPARγ agonist, was significantly suppressed in Liver mt PPARγ Tg (Fig. 4C). These data provide supporting evidence for the dominant-negative effect of the transgene in vivo.

At the age of 10 weeks, Liver mt PPARγ Tg showed a mean body weight comparable to their nontransgenic littermates (non-Tg). The plasma parameters for glucose and lipid metabolism were comparable between the genotypes (Table 1). The liver weight, histology, and triglyceride content were not altered in the transgenics (Table 1, Fig. 5). Because our goal was to elucidate the impact of mutant PPARγ in steatosis, we next subjected mice to 2 distinct steatogenic stimuli, prolonged fasting and dietary lipid overload.

3.4. Liver mt PPARγ Tg are susceptible to fasting-induced steatosis

Both Liver mt PPARγ Tg and non-Tg developed steatosis as a result of prolonged food deprivation for 72 hours (Fig. 5A). Of note, in the fasted state, the steatosis was drastically aggravated in Liver mt PPARγ Tg in comparison with non-Tg. In non-Tg, microvesicular steatosis was found, localized around the central venule. In contrast, macrovesicular, as well as microvesicular, steatosis was prominent and far-reaching toward the portal area in Liver mt PPARγ Tg (Fig. 5A) (n = 5, representative data). The vacuoles visualized upon H-E staining were positive for lipid by oil red O staining (data not shown). In addition to these histological findings, the liver triglyceride content was also significantly higher in the transgenics (Fig. 5B) (n = 5). Worsening of fasting-induced steatosis in Liver mt PPARγ Tg is reminiscent of PPARα knockout mice, which exhibit increased susceptibility to fasting-induced steatosis [14]. It can be presumed that the worsening of fasting-induced steatosis in Liver mt PPARγ Tg is attributable to PPARα suppression in the liver. Augmented AOX and CPT-I expression in the fasted liver was significantly suppressed in Liver mt PPARγ Tg (Fig. 5C), further supporting a role for PPARα suppression in the severe steatosis in the fasted Liver mt PPARγ Tg.

3.5. Liver mt PPARγ Tg are protected against high-fat diet–induced steatosis

Hepatic expressions of PPARα and PPARγ were substantially increased in HFD-induced steatosis (Fig. 1). To

Table 1
Body weight, liver weight, and plasma parameters of Liver mt PPARγ Tg mice

Genotype	Body weight (g)	Liver weight (g)	ALT (IU/L)	Glucose (mmol/L)	Insulin (ng/mL)	TG (mg/dL)	NEFA (mEq/L)
Wild type	21.4 ± 1.2	1.49 ± 0.03	28.0 ± 4.7	8.0 ± 1.2	1.03 ± 0.13	106.9 ± 23.7	767.5 ± 82.4
Liver mt PPARγ Tg	21.8 ± 2.4	1.48 ± 0.01	26.5 ± 8.0	7.7 ± 1.1	0.98 ± 0.15	97.98 ± 30.4	709.0 ± 117

Ten-week-old male wild-type (n = 5) and Liver mt PPARγ Tg mice (n = 5) maintained on a chow diet were used. Blood was extracted 9:00 to 11:00 AM from mice fed ad libitum. Data are mean ± SEM. No significant difference was observed between the genotypes. ALT indicates alanine aminotransferase; TG, triglyceride; NEFA, nonesterified fatty acids.

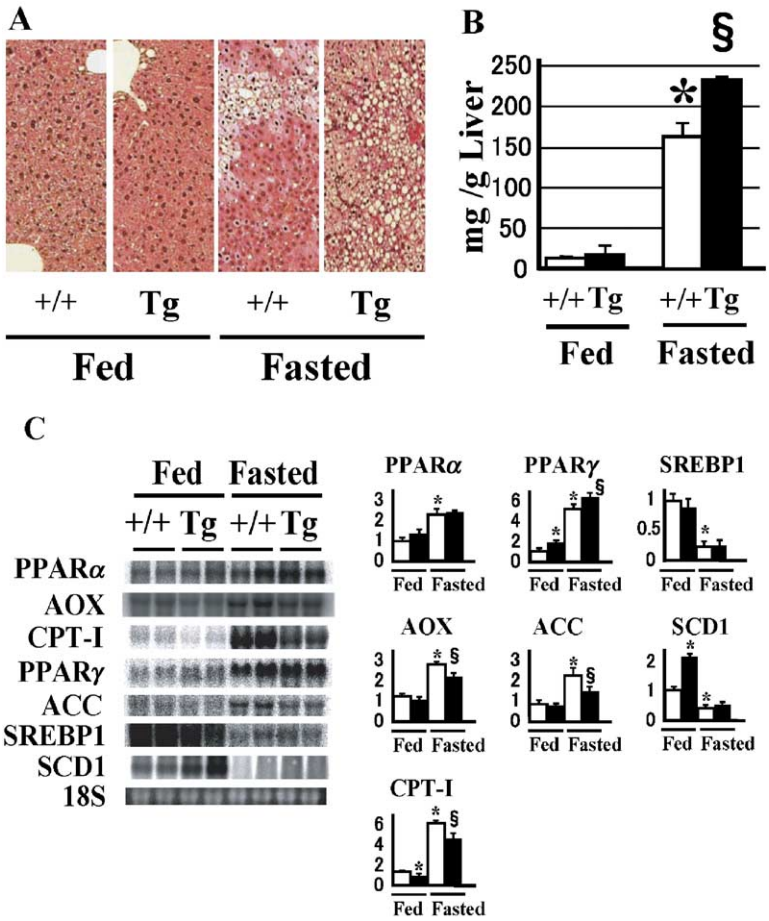

Fig. 5. Liver histology (A) (H-E, magnification ×200; representative data), liver triglyceride content (B), and mRNA levels for a series of PPAR target genes (C) in fasted Liver mt PPARγ Tg (Tg) and nontransgenic littermates (+/+). C, Representative blots and quantified data normalized by the 18S rRNA expression level (in arbitrary units) are shown. Open bars, +/+; dark bars, Liver mt PPARγ Tg (n = 5 for each group), *P < .05 vs Fed +/+, §P < .05 vs Fasted +/+.

elucidate the functional relevance of increased PPARα and PPARγ expressions in HFD-induced steatosis, the liver histology and triglyceride content were analyzed in HFD-fed Liver mt PPARγ Tg. In striking contrast to fasting-induced steatosis, HFD-induced steatosis was markedly ameliorated in Liver mt PPARγ Tg compared with non-Tg (Fig. 6A) (n = 5, representative data). In HFD-fed non-Tg, diffuse and severe macrovesicular steatosis was observed. On the other hand, high-fat feeding resulted only in limited numbers of lipid droplets in perivenular regions in the liver of Liver mt PPARγ Tg (Fig. 6A). In parallel with the histological findings, hepatic triglyceride content was also significantly lowered in Liver mt PPARγ Tg (Fig. 6B) (n = 5). In the transgenics, hepatic expression of both of the PPARα targets, AOX and CPT-I, was suppressed on HFD (Fig. 6C). AOX and CPT-I are critical factors in fatty acid oxidation in the liver, and down-regulation of AOX and CPT-I in PPARα knockout mice is associated with aggravated HFD-induced steatosis [14,21,24]. Liver mt PPARγ Tg are different from PPARα knockout mice in that PPARγ is also suppressed. The mRNA level for ACC, a PPARγ target gene in the liver, was also suppressed in Liver mt PPARγ Tg even on HFD (Fig. 6C). ACC is a representative lipogenic enzyme, and its suppression in the transgenics may partly explain the ameliorated steatosis in HFD-fed Liver mt PPARγ Tg.

3.6. Concerted up-regulation of PPARα and PPARγ target genes in steatosis is abrogated in Liver mt PPARγ Tg

In the wild-type mice, both the PPARα and PPARγ mRNA levels were augmented in both fasting- and HFD-induced steatosis (Fig. 1). Accordingly, the AOX, CPT-I, and ACC mRNA levels were coordinately elevated as a result of fasting and HFD (Figs. 5C and 6C). In contrast, hepatic expression of both sterol regulatory element binding protein 1 (SREBP1) and stearoyl-CoA desaturase 1 (SCD1) was decreased in fasting-induced steatosis, whereas it was augmented in HFD-induced steatosis (Figs. 5C and 6C). These findings suggest distinct molecular mechanisms underlying fasting- and HFD-induced steatosis as far as SREBP1 and SCD1 are concerned.

Expression of SCD1, a potent lipogenic enzyme [41], was augmented in chow-fed Liver mt PPARγ Tg without histological or biochemical signs of steatosis (Fig. 5A and C). SCD1 is a target of PPARα [42], PPARγ [15], and SREBP1 [43] in the liver. The mechanisms whereby

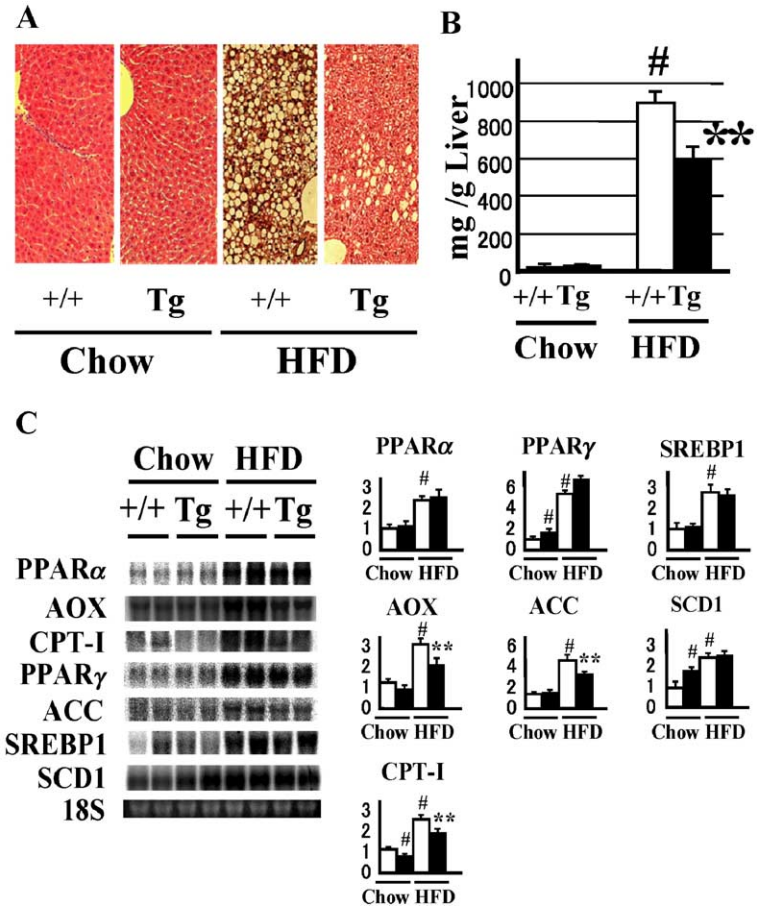

Fig. 6. Liver histology (A) (H-E, magnification ×200; representative data), liver triglyceride content (B), and mRNA levels for a series of PPAR target genes (C) in high-fat diet (HFD)–fed mice. C, Representative blots and quantified data normalized by the 18S rRNA expression level (in arbitrary units) are shown. Open bars, +/+; dark bars, Liver mt PPARγ Tg (n = 5 for each group), #$P < .05$ vs Chow-fed +/+, **$P < .05$ vs HFD-fed +/+.

SCD1 is up-regulated in Liver mt PPARγ Tg must await further investigation.

In Liver mt PPARγ Tg, the AOX, CPT-I, and ACC mRNA levels were all suppressed by fasting as well as HFD (Figs. 5C and 6C). Similar suppression of these genes in Liver mt PPARγ Tg by fasting and HFD does not provide a mechanistic explanation for the different hepatic outcomes as a result of fasting and HFD. On the other hand, suppression of the PPARα and PPARγ target genes in the transgenics in the fasted and HFD-fed states supports the dominant-negative effect of the transgene even in the case of dietary modification. Although the molecular mechanisms still need to be investigated, the distinct responses of Liver mt PPARγ Tg to the 2 types of steatogenic stimuli indicate that PPARα and PPARγ are differently involved in the pathophysiology of fasting- and HFD-induced steatosis.

4. Discussion

To explore the impact of PPARα and PPARγ cosuppression in the liver, we generated liver-specific mutant PPARγ transgenic mice (Liver mt PPARγ Tg), in which the transcriptional activities of liver PPARα and PPARγ are substantially suppressed. Liver mt PPARγ Tg was susceptible to fasting-induced steatosis, whereas HFD-induced steatosis was alleviated in the transgenics.

PPARα is highly expressed in the liver and brown adipose tissue in mice [14]. PPARγ is preferentially expressed in adipocytes but is also present in the liver, and its expression is augmented in murine steatosis [27-30]. It has been reported that, in liver tissue, PPARα and PPARγ are expressed at higher levels in hepatocytes than in endothelial and Kupffer cells [44]. Our data show that PPARα and PPARγ expressions are augmented in both fasting- and HFD-induced steatotic liver, leading us to speculate that cosuppression of PPARα and PPARγ in the liver might have some effects on the development of steatosis.

L468A/E471A mutant PPARγ1 has been reported to serve as a dominant-negative on PPARγ1 and γ2 activities, by recruiting and binding transcriptional corepressors [32]. In addition, it has also been shown that, in JEG-3 trophoblastic cells, L468A/E471A mutant PPARγ1 exerts an inhibitory effect on PPARα transcriptional activity [32]. In our experiment using HEK293 cells, L468A/E471A mutant PPARγ1 exhibited a striking dominant-negative effect on both PPARα and PPARγ1.

It is hard to assess exactly a dominant-negative effect in vivo because numerous factors such as putative endogenous ligands or coregulators are involved in the regulation of PPARα and PPARγ activities [45]. In Liver mt PPARγ Tg, the augmentation of the mRNA levels for AOX and ACC by respective treatment with agonists for PPARα and PPARγ was attenuated. CPT-I expression was significantly decreased in both the presence and absence of the PPARα agonist. Decreased expression of such target genes provides in vivo evidence that transgene-derived L468A/E471A mutant PPARγ1 exerts its dominant-negative effect on both PPARα and PPARγ. Suppression of the target genes in Liver mt PPARγ Tg was also evident in the fasted and HFD-fed conditions. The extent of target gene suppression in vivo was small compared with the reduction in PPRE-luciferase activity in vitro. The difference may partly be explained by regulatory mechanisms for AOX, CPT-I, and ACC independent of PPARα and PPARγ.

A growing body of evidence indicates that lipid accumulation in the liver is associated with insulin resistance [8,9]. The hepatic lipid content is determined by the balance between tissue uptake/synthesis and release/degradation of lipids [13]. PPARα is a key regulator of lipid metabolism in the liver, playing a crucial role in fatty acid oxidation and degradation [14,21-24]. Notably, mainly through enhancement of fatty acid oxidation, treatment with PPARα agonists improved steatosis in lipoatrophic A-ZIP/F-1 mice [46] and ethanol-fed mice [47]. Liver-specific PPARγ disruption has been shown to improve severe fatty liver in *ob*/*ob* mice [15], with reduced expression of a set of PPARγ target genes involved in lipid synthesis and uptake. Adenoviral overexpression of wild-type PPARγ1 in the liver augments expression of lipogenic enzymes and results in steatosis [31]. These reports indicate that liver PPARα is antisteatotic, whereas liver PPARγ is prosteatotic.

Liver mt PPARγ Tg exhibit opposite responses to fasting and HFD in terms of their susceptibility to steatosis. Enhancement of the mRNA levels for AOX and CPT-I due to fasting was attenuated in the transgenics, raising the possibility that suppression of PPARα-dependent fatty acid oxidation may account for the aggravation of steatosis in Liver mt PPARγ Tg (Fig. 7). In contrast, the augmented ACC expression on HFD was attenuated in the transgenics, indicating that suppression of PPARγ-induced lipogenesis may contribute to the amelioration of steatosis in Liver mt PPARγ Tg (Fig. 7). However, in fasted Liver mt PPARγ Tg, ACC expression was also suppressed compared with fasted non-Tg. Conversely, expression of antisteatotic AOX and CPT-I was reduced in HFD-fed transgenics. Further studies are necessary to elucidate the molecular mechanisms underlying the opposite responses of Liver mt PPARγ Tg to fasting and HFD.

In summary, we demonstrate that liver-specific expression of mutant PPARγ leads to suppression of PPARα and PPARγ target gene expression in vivo. Liver mt PPARγ Tg are susceptible to fasting-induced steatosis, but they are protected against HFD-induced steatosis, suggesting a possible interplay of PPARα and PPARγ in the pathophysiology of steatosis.

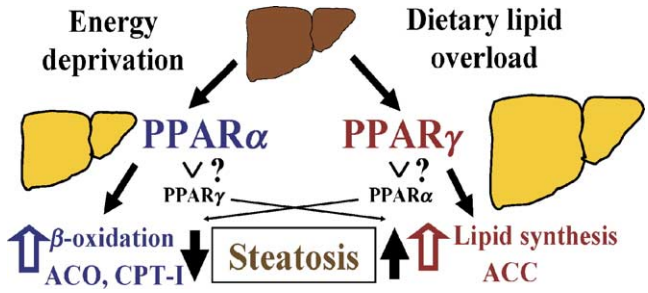

Fig. 7. Hypothetical scheme: possible distinct roles of PPARα and PPARγ in fasting-induced and high-fat diet–induced steatosis in mice. The induction of AOX and CPT-I mRNA levels due to fasting is attenuated in the Liver mt PPARγ Tg, suggesting the possibility that suppression of PPARα-dependent fatty acid oxidation may account for the aggravation of fasting-induced steatosis in the transgenics. In contrast, the augmented ACC expression on HFD is attenuated in the transgenics, indicating that the suppression of PPARγ-dependent lipogenesis may contribute to the amelioration of HFD-induced steatosis. The opposite hepatic outcomes in Liver mt PPARγ Tg as a result of fasting and high-fat feeding may indicate distinct roles of PPARα and PPARγ in 2 different types of nutritionally provoked steatosis.

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