HHS Public Access

Author manuscript

Alcohol Clin Exp Res. Author manuscript; available in PMC 2017 December 26.

Published in final edited form as:

Alcohol Clin Exp Res. 2016 May ; 40(5): 988–999. doi:10.1111/acer.13049.

Hepatic peroxisome proliferator-activated receptor gamma signaling contributes to alcohol-induced hepatic steatosis and inflammation in mice

Wenliang Zhang, Ph.D[1], **Qian Sun, M.S.**[1,2], **Wei Zhong, Ph.D**[1], **Xinguo Sun**[1], and **Zhanxiang Zhou, Ph.D**[1,2,2*]

[1]Center for Translational Biomedical Research, University of North Carolina at Greensboro, North Carolina Research Campus, Kannapolis, NC, USA 28081

[2]Department of Nutrition, University of North Carolina at Greensboro, North Carolina Research Campus, Kannapolis, NC, USA 28081

Abstract

Background—Peroxisome proliferator-activated receptor gamma (PPARγ) signaling has been shown to regulate lipogenesis and lipid accumulation. Previous studies have shown that hepatic PPARγ is up-regulated in steatotic liver of both animal and human. However, the effects of hepatic PPARγ signaling on alcoholic liver disease (ALD) remain elusive.

Methods—In order to determine the role of hepatic PPARγ signaling on ALD, wild type (WT) and hepatocyte-specific PPARγ knockdown (PPARγΔHep) mice were fed a modified Lieber-DeCarli alcohol or isocaloric maltose dextrin control liquid diet for 8 weeks to induce ALD. Blood parameters, hepatic steatosis, and inflammation were measured after 8-week alcohol feeding.

Results—Alcohol feeding to WT mice resulted in liver damage (alanine aminotransferase (ALT), 94.68 ± 17.05 U/L; aspartate aminotransferase (AST), 55.87 ± 11.29), which was significantly alleviated by hepatic PPARγ knockdown (ALT, 57.36 ± 14.98 U/L; AST, 38.06 ± 3.35). Alcohol feeding led to marked lipid accumulation and up-regulation of lipogenic genes including fatty acid transport protein 1 (FATP1), acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), lipin1, diacylglycerol acyltransferases 1 (DGAT1), and diacylglycerol acyltransferases 2 (DGAT2) in the livers of WT mice. Knockdown of hepatic PPARγ significantly alleviated alcohol-induced lipid accumulation and abolished the up-regulation of FASN, DGAT1, and DGAT2. Silencing of PPARγ in FL83B cells significantly decreased ethanol-, linoleic acid-, and ethanol plus linoleic acid-induced lipid accumulation. Knockdown of hepatic PPARγ also significantly reduced alcohol-induced inflammatory chemokine (monocyte chemotactic protein 1 (MCP1), keratinocyte-derived chemokine (KC), interferon gamma-induced protein 10 (IP-10)) and inflammatory infiltration (lymphocyte antigen 6 complex, locus G (Ly6G), and F4/80).

*Corresponding author: Zhanxiang Zhou, Center for Translational Biomedical Research and Department of Nutrition, University of North Carolina at Greensboro, North Carolina Research Campus, 500 Laureate Way, Suite 4226, Kannapolis, NC 28081. Phone: 704-250-5800. Fax: 704-250-5809. z_zhou@uncg.edu.

Conflict of Interest: None.

Conclusions—The results suggest that hepatic PPARγ signaling contributes to alcohol-induced liver injury by promoting hepatic steatosis and inflammation.

Keywords

alcoholic liver disease; peroxisome proliferator-activated receptor gamma; hepatic lipogenesis; hepatic inflammation

Introduction

Peroxisome proliferator-activated receptor gamma (PPARγ), a type II nuclear receptor, transcriptionally controls numerous cellular processes, including lipid metabolism, glucose homeostasis, and inflammation. It has been implicated in the development of many diseases including obesity, diabetes, atherosclerosis, and cancer (Guan and Breyer, 2001). Data showed that PPARγ is abundantly expressed in mature adipocytes and is elevated in the livers of animals that develop fatty livers (Schadinger et al., 2005). Up-regulation of PPARγ and its targeted gene, CD36, was observed in mouse liver as early as 2 weeks after high fat diet feeding (Inoue et al., 2005). PPARγ is also markedly elevated in steatotic livers of the A-ZIP/F-1 mice, a model of severe congenital lipoatrophic diabetes (Chao et al., 2000), and other mouse models of hepatic steatosis, including *ob/ob* model of diabetic mice and KKAy diabetic mice (Bedoucha et al., 2001, Rahimian et al., 2001). Moreover, PPARγ is also up-regulated in the liver of obese patients with nonalcoholic fatty liver disease (NAFLD) (Pettinelli and Videla, 2011), indicating that increased PPARγ is a general property of steatotic liver.

Alcohol consumption has long been known as a risk factor for both disease and death. Alcohol abuse has been causally related to more than 60 different medical conditions including alcoholic liver disease (ALD), which typically evolves through three progressive stages: steatosis (fatty liver), hepatitis, and cirrhosis (Corrao et al., 2004, Zhang et al., 2015, Beier et al., 2011). Chronic alcohol feeding contributes to the development of fatty liver through multiple mechanisms including increase of plasma corticosterone (Sun et al., 2013), induction of zinc deficiency (Zhong et al., 2013), hyperlipolysis of adipose tissue and reverse triglyceride (TG) transport to liver (Zhong et al., 2012), and induction of leptin deficiency (Tan et al., 2012). However, the effect of chronic alcohol consumption on hepatic PPARγ signaling and the role of hepatic PPARγ signaling pathway in ALD remain unclear and needs to be elucidated. In the present study, a mouse model of chronic alcohol consumption was designed to investigate the role of hepatic PPARγ signaling pathway in ALD.

Materials and Methods

Animals and alcohol feeding experiments

Albumin-Cre recombinase transgenic (Albumin-Cre Tg) mice and PPARγ$^{flox/flox}$ mice were purchased from The Jackson Laboratories (Bar Harbor, MA). The two lines were intercrossed to generate albumin-Cre/$^{+}$ PPARγ$^{flox/flox}$ mice (PPARγΔHep). Cre-negative animals were used as wild type (WT) controls. Eight-week-old male PPARγΔHep and WT

mice were pair-fed a modified Lieber-DeCarli alcohol (alcohol-fed, AF) or isocaloric maltose dextrin control (pair-fed, PF) liquid diet for 8 weeks (n = 8). The diet was purchased from Dyets Inc. (Bethlehem, PA). The ingredients of the diet can be found at http://dyets.com//?s=Lieber+DeCarli++Rat+Diet. In brief, the ethanol content (%, w/v) in the diet was start with 3 and gradually increased to 4.4. The amount of food given to the pair-fed mice was that the alcohol-fed mice consumed in the previous day. The animal protocol was approved by the Institutional Animal Care and Use Committee of the North Carolina Research Campus (13016). At the end of 8-week feeding, mice were anesthetized with inhalational isoflurane, epididymal white adipose tissue (EWAT) and livers were collected.

Determination of liver injury

Liver injury was determined by measuring plasma enzyme activity, liver pathology, and lipid accumulation. Plasma alanine aminotransferase (ALT) activity and aspartate aminotransferase (AST) activity were colorimetrically measured using Infinity ALT Reagent and Infinity AST Reagent, respectively (Thermo Scientific, Waltham, MA). Liver tissue paraffin sections were prepared and stained with hematoxylin and eosin (H-E). Quantitative assay of TG in liver tissue were measured by TG Quantification Colorimetric/Fluorometric Kit (BioVision, Milpitas, CA). Liver cholesterol concentrations were measured with the Infinity Cholesterol Reagent (Thermo Scientific, Waltham, MA).

Blood metabolites assay

Blood glucose was measured using a One-Touch Ultra2 blood glucose meter (Life Scan, Milpitas, CA). Plasma TG and cholesterol concentrations were measured with the Infinity TG Reagent and Infinity Cholesterol Reagent (Thermo Scientific, Waltham, MA), respectively. Concentrations of plasma free fatty acids (FFA) were determined with a FFA Quantification Kit (BioVision, Mountain View, CA).

Immunoblot analysis

Liver tissue proteins were extracted by T-PER tissue extraction reagent (Thermo scientific) containing protease inhibitors (Sigma-Aldrich, St. Louis, MO). Aliquots containing 40 μg proteins were loaded onto a 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE). After electrophoresis, proteins were transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was probed with polyclonal antibodies against PPARγ (Cell Signaling Technology, Danvers, MA), SREBP-1c (#LS-B93, LSBio, Seattle, WA) and β-Actin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), respectively. The membrane was then incubated with HRP-conjugated goat anti-rabbit IgG, or goat anti-mouse IgG antibody. The protein bands were visualized by an Enhanced Chemiluminescence detection system (GE Healthcare, Piscataway, NJ) and quantified by densitometry analysis.

Immunohistochemical staining

Liver tissue sections were rehydrated and incubated overnight at 4°C with anti-SREBP-1c antibody, followed by incubation with a horseradish peroxidase-conjugated secondary antibody (Thermo Scientific, Waltham, MA) for 30 min at room temperature. Visualization was conducted using diaminobenzidine as HRP substrate.

Silencing of PPARγ in FL83B mouse hepatocytes

Mouse FL83B hepatocytes (ATCC CRL-2390) from American Type Culture Collection (Rockville, MD, USA) were grown in ATCC-formulated F-12K Medium (Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Atlanta Biologicals, Lawrenceville, GA), 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen), at 37 °C in a humidified atmosphere of 5% CO_2. FL83B cells were transfected with PPARγ shRNA lentiviral particles (#sc-29456-v) or control shRNA lentiviral particles (#sc-108080) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) according to manufacture protocol. Stable clones expressing the shRNA were selected using Puromycin dihydrochloride (sc-108071, Santa Cruz Biotechnology, Inc., Santa Cruz, CA).

Determination of PPARγ effect on alcohol-induced lipid accumulation *in vitro*

FL83B-si-PPARγ and FL83B-si-Control cells were treatment with ethanol (100mM), linoleic acid (200μM), or ethanol plus linoleic acid for 48 hours. Cells were fixed with 10% formalin and processed with Oil Red O solution (#O1391, Sigma-Aldrich, St. Louis, MO) to stain neutral lipids.

qPCR analysis

Total RNA was isolated from liver with TRIzol reagent (Life Technologies, Grand Island, NY) and reverse transcribed with TaqMan Reverse Transcription Reagents (Applied Biosystems, Carlsbad, CA). The gene expression of related mRNA was measured in triplicate with SYBR green PCR Master Mix (Qiagen, Valencia, CA) by the comparative cycle threshold method using 7500 real-time PCR system (Applied Biosystems). The data were normalized to the geometric mean of mRNA levels of 18s rRNA, β-Actin, and 36B4 and presented as fold changes, setting the values of WT-PF mice as one (Vandesompele et al., 2002, Derveaux et al., 2010). The ΔΔCT method was used for data analysis to determine the fold differences in levels of expression. The forward and reverse primers shown in Table 1 were purchased from Integrated DNA Technologies (Coralville, IA).

Statistics

Results are expressed as mean ± standard deviation (SD). Data were analyzed by Andeson-Darling test. Results showed that all data were normally distributed. Differences between two groups were analyzed by two-tail Student's *t*-test. Differences among multiple groups were analyzed by two-way analysis of variance (ANOVA) followed by Bonferroni post-tests. Differences between groups were considered significant at $P < 0.05$.

Results

Chronic alcohol feeding induces hepatic steatosis and up-regulated PPARγ in mouse liver

To find out the effect of chronic alcohol consumption on hepatic PPARγ signaling, the expression levels of hepatic PPARγ after 8-week alcohol feeding were examined. H-E staining of liver section demonstrated that 8 weeks of alcohol feeding increased both the number and size of lipid droplets in the livers of WT mice (Figure 1A). Moreover,

immunoblot analysis showed that 8 weeks of alcohol feeding significantly elevated the expression levels of PPARγ in the livers of WT mice (Figure 1B).

Knockdown of PPARγ in hepatocytes attenuates alcohol-induced increase of liver to body weight ratio

Because PPARγ was up-regulated in alcoholic steatotic liver, PPARγΔHep mice were produced. Western blot analysis showed that hepatic PPARγ was successfully knocked down (Figures 2A, 2B). No significant difference was found before alcohol feeding in body weight and food consumption between WT and PPARγΔHep mice (data not shown). Next, WT and PPARγΔHep male mice were used to investigate the specific role of hepatic PPARγ signaling in the development of ALD. Eight weeks of alcohol feeding significantly lowered body weight (Figure 2C) and EWAT weight (Figure 2D), had no significant effect on liver weight (Figure 2E), but resulted in significant increase of liver to body weight ratio (Figure 2F) in WT mice. Knockdown of hepatic PPARγ did not affect the alcohol-induced changes of body weight or EWAT weight (Figures 2B-2C), but significantly attenuated alcohol-induced increase of liver to body weight ratio (Figure 2F) in PPARγΔHep mice.

Knockdown of hepatic PPARγ alleviates alcohol-induced liver injury

To determine if knockdown of PPARγ in hepatocytes alleviates hepatic injury, serum ALT and AST were measured. As shown in Figure 3, eight weeks of alcohol feeding induced significant increase of both ALT and AST in WT mice (Figures 3A-B). In contrast, the increase of ALT and AST was significantly inhibited by knockdown of PPARγ in hepatocytes (Figures 3A-B). Eight-week of alcohol feeding also significantly lowered blood cholesterol (Figure 3C) and glucose level (Figure 3D) in both WT and PPARγΔHep mice. But eight-week of alcohol feeding showed no effect on plasma TG (Figure 3E) or plasma FFA (Figure 3F) level in both WT and PPARγΔHep mice.

Knockdown of hepatic PPARγ alleviates alcohol-induced hepatic steatosis

To determine if knockdown of PPARγ in hepatocytes protects mice from ALD, PPARγ expression level was analyzed by western blot after 8-week alcohol feeding. Results showed that alcohol feeding significantly induced PPARγ expression in the liver of WT mouse. No significant induction of PPARγ was found in PPARγΔHep mice (Figures 4A-4B). H-E staining of liver sections demonstrated that 8 weeks of alcohol feeding significantly increased the number and size of lipid droplets in the liver of WT mice (Figure 4C). In the livers of PPARγΔHep mice, although the number and size of lipid droplets were also increased by 8 weeks of alcohol feeding, the increase was significantly alleviated compared to that of WT-AF mice (Figure 4C). In consistent with this, quantitative measurement of hepatic lipid contents showed that 8 weeks of alcohol feeding significantly increased hepatic TG accumulation in the liver of WT mice ($P < 0.05$) (Figure 4D). In contrast, alcohol-induced TG accumulation was significantly alleviated in the liver of PPARγΔHep-AF mice (Figure 4D). Quantitative measurement of hepatic cholesterol showed that 8 weeks of alcohol feeding significantly increased hepatic cholesterol levels in both WT and PPARγΔHep mice (Figure 4E).

Knockdown of hepatic PPARγ inhibits alcohol-induced lipogenic gene expression in mice liver

To find out how PPARγΔHep mice were protected from alcohol induced TG accumulation and hepatic steatosis, the mRNA levels of genes related to lipid metabolism were measured. As shown in Figure 5, eight weeks of alcohol feeding to WT mice induced gene expression of hepatic fatty acid transport protein 1 (FATP1) (Figure 5A), cytochrome P450 4A (CYP4A) (Figure 5B), Acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN) (Figure 5C), LIPIN1, diglyceride acyltransferase-1 (DGAT1), and diglyceride acyltransferase-2 (DGAT2) (Figure 5D); decreased gene expression of hepatic fatty acid transport protein 2 (FATP2), fatty acid binding protein 1 (FABP1), lipoprotein lipase (LPL), very low density lipoprotein receptor (VLDLR) (Figure 5A), carnitine palmitoyltransferase 1α (CPT1α), Acyl-CoA Oxidase 1 (ACOX1) (Figure 5B), and microsomal triglyceride transfer protein (MTTP) (Figure 5E). In contrast, knockdown of PPARγ in hepatocytes abolished alcohol induced up-regulation of FASN, DGAT1, and DGAT2 (Figures 5C-D), but showed no effect on FATP1, FATP2, FABP1, LPL, VLDLR, CPT1α, ACOX1, CYP4A, ACC, Lipin1, or MTTP. Other genes including hepatic cluster of differentiation (CD36), fatty acid transport protein 5 (FATP5), acyl-CoA synthetase long-chain family member 1 (ACSL1) (Figure 5A), long Chain acyl-CoA dehydrogenase (ACADL) (Figure 5B), ATP citrate lyase (ACL) (Figure 5C), glycerol-3-phosphate acyltransferase (GPAT1), 1-acylglycerol-3-phosphate O-acyltransferase 1 (AGPAT1) (Figure 5D), and apolipoprotein B (ApoB) (Figure 5E), showed no response to alcohol feeding in either WT or PPARγΔHep mice.

Knockdown of hepatic PPARγ inhibits alcohol-induced activation of SREBP-1c

Immunohistochemical staining of liver section was used to investigate the effect of hepatic PPARγ knockdown on the cooperative action of PPARγ and SREBP-1c. As showed in Figure 6A, 8-week alcohol feeding significantly induced the nuclear translocation of SREBP-1c in WT mouse liver. In PPARγΔHep mouse liver, however, alcohol-feeding-induced nuclear translocation of SREBP-1c was abrogated. Western blot results showed that 8-week alcohol feeding significantly induced both precursor SREBP-1c and cleaved SREBP-1c. The activation of SREBP-1c was abolished by hepatic PPARγ knockdown (Figures 6B-D).

To determine if knockdown of PPARγ in hepatocytes affects alcohol-induced hepatic inflammation, inflammatory infiltration markers and inflammatory chemokines were measured by qPCR analysis after eight weeks of alcohol feeding. As shown in Figure 7, chronic alcohol feeding for 8 weeks significantly increased the expression of inflammatory markers, including monocyte chemotactic protein 1 (MCP1), keratinocyte-derived chemokine (KC), interferon gamma-induced protein 10 (IP-10) (Figure 7A), lymphocyte antigen 6 complex, locus G (Ly6G), and F4/80 (Figure 7B) in the livers of WT mice. However, the alcohol-induced up-regulation of inflammatory markers was significantly inhibited or normalized in PPARγΔHep-AF mice. In the livers of PPARγΔHep-AF mice, no up-regulation of MCP1, Ly6G, or F4/80 was found. The alcohol induced up-regulation of IP-10 and KC was significantly lowered than that of WT-AF mice, suggesting that knockdown of PPARγ in hepatocytes abolished alcohol induced hepatic inflammation.

Cluster of Differentiation 68 (CD68) showed on response to alcohol feeding in either WT or PPARγΔHep mice.

Silencing of PPARγ inhibits alcohol-induced lipid accumulation *in vitro*

PPARγ was successfully silenced in FL83B cells using PPARγ shRNA lentiviral particles (Figures 8A-B). Oil Red O staining showed that ethanol treatment, linoleic acid treatment, and ethanol plus linoleic acid treatment induced significant lipid accumulation in FL83B-si-control cells (Figure 8C). In contrast, silencing of PPARγ significantly alleviated ethanol, linoleic acid, and linoleic acid plus ethanol induced lipid accumulation in FL83B-si- PPARγ cells (Figure 8C).

Discussion

PPARs (isoforms α, δ, and γ) are transcription factors belonging to the superfamily of nuclear receptors. PPARγ signaling plays a very important role in the regulation of fatty acid storage (adipose tissue expansion) and glucose metabolism (Smith, 2002, Medina-Gomez et al., 2007). The present study shows that hepatic PPARγ was significantly up-regulated in alcoholic steatotic liver and knockdown of PPARγ in hepatocytes alleviated alcohol-induced hepatic injury. The protective effect of hepatic PPARγ knockdown on alcohol-induced hepatic injury was associated with both attenuation of alcohol-induced hepatic steatosis and alleviation of alcohol-induced hepatic inflammation. These data demonstrates, for the first time, that PPARγ signaling in hepatocytes promotes alcohol-induced hepatic steatosis, inflammatory cell infiltration, inflammatory chemokine production, and liver injury.

It is well-documented that as a nuclear hormone receptor, PPARγ is necessary and sufficient for *in vitro* adipogenesis and transcriptionally activates numerous adipogenic and lipogenic genes important for lipid storage, lipid synthesis, and glucose sensing (Chawla et al., 1994, Barak et al., 1999, He et al., 2003). A number of studies have demonstrated that enhanced expression of PPARγ and lipogenic genes in steatotic liver of animal models or patients with NAFLD (Gavrilova et al., 2003, Matsusue et al., 2003). However, it was unclear that whether PPARγ signaling specifically in hepatocytes contributes to the progression of alcohol-induced hepatic steatosis. The present study shows that the expression of hepatic PPARγ was significantly enhanced in alcohol-induced steatotic liver of AF group compared to that of PF group, which clearly demonstrates that hepatic PPARγ plays a role in the progression of ALD.

To further elucidate the role of hepatic PPARγ signaling in the progression of ALD, PPARγ was knocked down in hepatocytes to generate PPARγΔHep mice. Then, WT and PPARγΔHep mice were fed with modified Lieber-DeCarli alcohol or control liquid diet for 8 weeks. Knockdown of PPARγ signaling in hepatocytes significantly attenuated alcohol-induced up-regulation of liver to body weight ratio, number and size of lipid droplets, hepatic TG level, and levels of ALT and AST compared to that of WT-PF mice. These data suggest that PPARγ signaling in hepatocytes promoted alcohol-induced hepatic steatosis and hepatic injury, and knockdown of hepatic PPARγ protected mice against alcohol-induced hepatic steatosis and injury.

It has been shown that the expression of PPARγ2 in perigonadal adipose tissues were significantly higher in female rats than in males, indicating that there are sex differences in PPARγ expression (Kadowaki et al., 2007). Estrogen receptor (ER) beta has been shown to negatively regulate ligand-induced PPAR signaling both *in vitro* and *in vivo* (Foryst-Ludwig et al., 2008, Wang and Kilgore, 2002). It also has been shown that the mass of parametrial white adipose tissue and expression of PPARγ in diestrus were significantly higher compared with estrus (Gui et al., 2006). In order to avoid the effect of estrogen or menstrual cycle on PPARγ signaling, only male mice were included in this study.

Previous studies demonstrated that genes activated by PPARγ stimulate lipid uptake and adipogenesis by fat cells (Jones et al., 2005), and dysregulation of lipid metabolism contributes to the development of alcoholic fatty liver (Zhong et al., 2013, Sun et al., 2013). In this study, we found that alcohol feeding dysregulated many pathways related to lipid metabolism, including fatty acid transportation/activation, fatty acid oxidation, fatty acid synthesis, TG synthesis, and VLDL secretion in both WT and PPARγΔHep mice. PPARγ knockdown in hepatocytes inhibited alcohol-induced up-regulation of FASN and DGAT2 and decreased DGAT1. As the enzyme catalyzes the last step in fatty acid biosynthesis, FASN is believed to be a major determinant of the maximal hepatic capacity to generate fatty acids by de novo lipogenesis (Dorn et al., 2010) and has been found elevated in patients and animals with NAFLD (Dorn et al., 2010, D'Souza A et al., 2012). The finding that PPARγ knockdown in hepatocytes attenuates alcohol-induced FASN up-regulation indicates that hepatic PPARγ signaling activates de novo lipogenesis to contribute to ALD. It is also known that fatty liver disease is characterized by accumulation of TG and other lipids in the liver (Villanueva et al., 2009). The final step in TG synthesis is catalyzed by DGAT enzymes, DGAT1 and DGAT2 (Cases et al., 1998, Cases et al., 2001). Increased levels of DGAT1 have been found in human livers with NAFLD (Kohjima et al., 2007). Mice lacking Dgat1 ($Dgat1^{-/-}$) have reduced tissue TG levels (Smith et al., 2000, Chen et al., 2002). Suppression of DGAT2 reversed diet-induced hepatic steatosis in animals (Choi et al., 2007). In the present study, both DGAT1 and DGAT2 were increased by alcohol feeding in the livers of WT mice. However, knockdown of PPARγ in hepatocytes abolished alcohol-induced up-regulation of DGAT2 and even decreased the expression of DGAT1, indicating that hepatic PPARγ signaling promotes hepatic TG synthesis to contribute to ALD. Silence of PPARγ in FL83B cells significantly inhibited ethanol-, linoleic acid-, or ethanol plus linoleic acid-induced lipid accumulation (Figure 8) further supported the concept that hepatic PPARγ signaling promotes hepatic TG synthesis to contribute to ALD.

Sterol regulatory element-binding protein 1c (SREBP-1c), a key regulator of lipogenesis, has been shown to transcriptionally regulate genes dedicated to the synthesis, oxidation, uptake, and transport of cholesterol, fatty acids, triglycerides, and phospholipids (Sakakura et al., 2001, Li et al., 2014). SREBP1 has been found to activate PPARγ through production of endogenous ligand (Kim et al., 1998). On the other hand, PPARγ has also been shown to regulate the expression of SREBP-1 (Schadinger et al., 2005). In order to investigate the effect of hepatic PPARγ knockdown and alcohol feeding on SREBP1, immunohistochemical staining of SREBP-1c was performed with liver sections. Results (Figure 6) showed that alcohol induced the expression of both precursor SREBP-1c and cleaved SREBP-1c in WT mice. Alcohol feeding also induced nuclear translocation of

SREBP-1c in WT mice. Hepatic PPARγ knockdown, however, abrogated alcohol-induced activation and nuclear translocation of SREBP-1c, suggesting that hepatic PPARγ knockdown might protect mice from ALD through inhibition of SREBP-1c activation and subsequent SREBP-1c-mediated lipogenic process in mouse liver.

A variety of PPARγ agonists had been used to treat diabetes (Kumar et al., 1996, Mayerson et al., 2002). It has been shown that application of PPARg agonists reduced alcohol-induced hepatic steatosis (Enomoto et al., 2003, Neuschwander-Tetri et al., 2003), which seems inconsistent with present study. However, it is known that PPAR-γ is primarily expressed in adipose tissue, so the major target of PPAR-γ agonists is adipose tissue. The restore of insulin sensitivity and the alleviation of hepatic steatosis by PPAR-γ agonists have been attributed to the direct effects of PPAR agonists on lipid metabolism in adipose tissue and to secondary effects on lipid and glucose metabolism in liver and skeletal muscle (Jiang et al., 2002, Way et al., 2001, Staels and Fruchart, 2005). Our group had showed that application of PPAR-γ agonists restored the lipid storage function of white adipose tissue and improved lipid homeostasis at the white adipose tissue-liver axis which were impaired by alcohol feeding to alleviate or abrogate alcoholic fatty liver (Zhong et al., 2012, Sun et al., 2012).

Accumulating evidence reveal that inflammation is strongly related to both NAFLD and ALD (Kwon et al., 2014, Zhong et al., 2013). Cytokines and chemokines play a pivotal role in inflammatory processes. MCP1, IP-10, and KC have been shown to play a role in hepatic inflammation or injury (Mandrekar et al., 2011, Reiberger et al., 2008, Sermon et al., 2003). Our and other groups have shown that alcohol feeding induces MCP1, IP-10, KC, Ly6G, and F4/80 (Sun et al., 2012, Zhong et al., 2013, Duly et al., 2015). However, the underlining mechanisms remain unclear. This study shows that knockdown of hepatic PPARγ attenuates alcohol-induced hepatic inflammation, suggesting that PPARγ signaling in hepatocytes contributes to alcohol-induced hepatic inflammation by promoting inflammatory cell infiltration and inflammatory chemokine release.

In summary, the results from the present study revealed that PPARγ signaling in hepatocytes contributes to hepatic steatosis by promoting lipid accumulation and hepatic inflammation through enhancing inflammatory chemokine release and inflammatory cell infiltration in ALD. These data provide a better understanding of hepatic PPARγ in the pathogenesis of ALD and suggest that targeting hepatic PPARγ signaling could be a therapy of ALD.

Acknowledgments

This research was supported by the National Institutes of Health (R01AA018844 and R01AA020212).

Sources of support: This study was supported by the National Institutes of Health (R01AA020212 and R01AA018844).

References

Barak Y, Nelson MC, Ong ES, Jones YZ, Ruiz-Lozano P, Chien KR, Koder A, Evans RM. PPAR gamma is required for placental, cardiac, and adipose tissue development. Molecular cell. 1999; 4:585–595. [PubMed: 10549290]

Bedoucha M, Atzpodien E, Boelsterli UA. Diabetic KKAy mice exhibit increased hepatic PPARgamma1 gene expression and develop hepatic steatosis upon chronic treatment with antidiabetic thiazolidinediones. J Hepatol. 2001; 35:17–23. [PubMed: 11495036]

Beier JI, Arteel GE, McClain CJ. Advances in alcoholic liver disease. Current gastroenterology reports. 2011; 13:56–64. [PubMed: 21088999]

Cases S, Smith SJ, Zheng YW, Myers HM, Lear SR, Sande E, Novak S, Collins C, Welch CB, Lusis AJ, Erickson SK, Farese RV Jr. Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. Proceedings of the National Academy of Sciences of the United States of America. 1998; 95:13018–13023. [PubMed: 9789033]

Cases S, Stone SJ, Zhou P, Yen E, Tow B, Lardizabal KD, Voelker T, Farese RV Jr. Cloning of DGAT2, a second mammalian diacylglycerol acyltransferase, and related family members. The Journal of biological chemistry. 2001; 276:38870–38876. [PubMed: 11481335]

Chao L, Marcus-Samuels B, Mason MM, Moitra J, Vinson C, Arioglu E, Gavrilova O, Reitman ML. Adipose tissue is required for the antidiabetic, but not for the hypolipidemic, effect of thiazolidinediones. J Clin Invest. 2000; 106:1221–1228. [PubMed: 11086023]

Chawla A, Schwarz EJ, Dimaculangan DD, Lazar MA. Peroxisome proliferator-activated receptor (PPAR) gamma: adipose-predominant expression and induction early in adipocyte differentiation. Endocrinology. 1994; 135:798–800. [PubMed: 8033830]

Chen HC, Smith SJ, Ladha Z, Jensen DR, Ferreira LD, Pulawa LK, McGuire JG, Pitas RE, Eckel RH, Farese RV Jr. Increased insulin and leptin sensitivity in mice lacking acyl CoA:diacylglycerol acyltransferase 1. J Clin Invest. 2002; 109:1049–1055. [PubMed: 11956242]

Choi CS, Savage DB, Kulkarni A, Yu XX, Liu ZX, Morino K, Kim S, Distefano A, Samuel VT, Neschen S, Zhang D, Wang A, Zhang XM, Kahn M, Cline GW, Pandey SK, Geisler JG, Bhanot S, Monia BP, Shulman GI. Suppression of diacylglycerol acyltransferase-2 (DGAT2), but not DGAT1, with antisense oligonucleotides reverses diet-induced hepatic steatosis and insulin resistance. The Journal of biological chemistry. 2007; 282:22678–22688. [PubMed: 17526931]

Corrao G, Bagnardi V, Zambon A, La Vecchia C. A meta-analysis of alcohol consumption and the risk of 15 diseases. Prev Med. 2004; 38:613–619. [PubMed: 15066364]

D'Souza AM, Beaudry JL, Szigiato AA, Trumble SJ, Snook LA, Bonen A, Giacca A, Riddell MC. Consumption of a high-fat diet rapidly exacerbates the development of fatty liver disease that occurs with chronically elevated glucocorticoids. Am J Physiol Gastrointest Liver Physiol. 2012; 302:G850–863. [PubMed: 22268100]

Derveaux S, Vandesompele J, Hellemans J. How to do successful gene expression analysis using real-time PCR. Methods. 2010; 50:227–230. [PubMed: 19969088]

Dorn C, Riener MO, Kirovski G, Saugspier M, Steib K, Weiss TS, Gabele E, Kristiansen G, Hartmann A, Hellerbrand C. Expression of fatty acid synthase in nonalcoholic fatty liver disease. International journal of clinical and experimental pathology. 2010; 3:505–514. [PubMed: 20606731]

Duly AM, Alani B, Huang EY, Yee C, Haber PS, McLennan SV, Seth D. Effect of multiple binge alcohol on diet-induced liver injury in a mouse model of obesity. Nutrition & diabetes. 2015; 5:e154. [PubMed: 25915743]

Enomoto N, Takei Y, Hirose M, Konno A, Shibuya T, Matsuyama S, Suzuki S, Kitamura KI, Sato N. Prevention of ethanol-induced liver injury in rats by an agonist of peroxisome proliferator-activated receptor-gamma, pioglitazone. J Pharmacol Exp Ther. 2003; 306:846–854. [PubMed: 12805475]

Foryst-Ludwig A, Clemenz M, Hohmann S, Hartge M, Sprang C, Frost N, Krikov M, Bhanot S, Barros R, Morani A, Gustafsson JA, Unger T, Kintscher U. Metabolic actions of estrogen receptor beta (ERbeta) are mediated by a negative cross-talk with PPARgamma. PLoS genetics. 2008; 4:e1000108. [PubMed: 18584035]

Gavrilova O, Haluzik M, Matsusue K, Cutson JJ, Johnson L, Dietz KR, Nicol CJ, Vinson C, Gonzalez FJ, Reitman ML. Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. The Journal of biological chemistry. 2003; 278:34268–34276. [PubMed: 12805374]

Guan Y, Breyer MD. Peroxisome proliferator-activated receptors (PPARs): novel therapeutic targets in renal disease. Kidney international. 2001; 60:14–30. [PubMed: 11422732]

Gui Y, Cai Z, Silha JV, Murphy LJ. Variations in parametrial white adipose tissue mass during the mouse estrous cycle: relationship with the expression of peroxisome proliferator-activated receptor-gamma and retinoic acid receptor-alpha. Canadian journal of physiology and pharmacology. 2006; 84:887–892. [PubMed: 17111033]

He W, Barak Y, Hevener A, Olson P, Liao D, Le J, Nelson M, Ong E, Olefsky JM, Evans RM. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100:15712–15717. [PubMed: 14660788]

Inoue M, Ohtake T, Motomura W, Takahashi N, Hosoki Y, Miyoshi S, Suzuki Y, Saito H, Kohgo Y, Okumura T. Increased expression of PPARgamma in high fat diet-induced liver steatosis in mice. Biochem Biophys Res Commun. 2005; 336:215–222. [PubMed: 16125673]

Jiang G, Dallas-Yang Q, Li Z, Szalkowski D, Liu F, Shen X, Wu M, Zhou G, Doebber T, Berger J, Moller DE, Zhang BB. Potentiation of insulin signaling in tissues of Zucker obese rats after acute and long-term treatment with PPARgamma agonists. Diabetes. 2002; 51:2412–2419. [PubMed: 12145152]

Jones JR, Barrick C, Kim KA, Lindner J, Blondeau B, Fujimoto Y, Shiota M, Kesterson RA, Kahn BB, Magnuson MA. Deletion of PPARgamma in adipose tissues of mice protects against high fat diet-induced obesity and insulin resistance. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102:6207–6212. [PubMed: 15833818]

Kadowaki K, Fukino K, Negishi E, Ueno K. Sex differences in PPARgamma expressions in rat adipose tissues. Biological & pharmaceutical bulletin. 2007; 30:818–820. [PubMed: 17409529]

Kim JB, Wright HM, Wright M, Spiegelman BM. ADD1/SREBP1 activates PPARgamma through the production of endogenous ligand. Proceedings of the National Academy of Sciences of the United States of America. 1998; 95:4333–4337. [PubMed: 9539737]

Kohjima M, Enjoji M, Higuchi N, Kato M, Kotoh K, Yoshimoto T, Fujino T, Yada M, Yada R, Harada N, Takayanagi R, Nakamuta M. Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. Int J Mol Med. 2007; 20:351–358. [PubMed: 17671740]

Kumar S, Boulton AJ, Beck-Nielsen H, Berthezene F, Muggeo M, Persson B, Spinas GA, Donoghue S, Lettis S, Stewart-Long P. Troglitazone, an insulin action enhancer, improves metabolic control in NIDDM patients. Troglitazone Study Group. Diabetologia. 1996; 39:701–709. [PubMed: 8781766]

Kwon HJ, Won YS, Park O, Chang B, Duryee MJ, Thiele GE, Matsumoto A, Singh S, Abdelmegeed MA, Song BJ, Kawamoto T, Vasiliou V, Thiele GM, Gao B. Aldehyde dehydrogenase 2 deficiency ameliorates alcoholic fatty liver but worsens liver inflammation and fibrosis in mice. Hepatology. 2014; 60:146–157. [PubMed: 24492981]

Li X, Li Y, Yang W, Xiao C, Fu S, Deng Q, Ding H, Wang Z, Liu G, Li X. SREBP-1c overexpression induces triglycerides accumulation through increasing lipid synthesis and decreasing lipid oxidation and VLDL assembly in bovine hepatocytes. The Journal of steroid biochemistry and molecular biology. 2014; 143:174–182. [PubMed: 24565561]

Mandrekar P, Ambade A, Lim A, Szabo G, Catalano D. An essential role for monocyte chemoattractant protein-1 in alcoholic liver injury: regulation of proinflammatory cytokines and hepatic steatosis in mice. Hepatology. 2011; 54:2185–2197. [PubMed: 21826694]

Matsusue K, Haluzik M, Lambert G, Yim SH, Gavrilova O, Ward JM, Brewer B Jr, Reitman ML, Gonzalez FJ. Liver-specific disruption of PPARgamma in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes. J Clin Invest. 2003; 111:737–747. [PubMed: 12618528]

Mayerson AB, Hundal RS, Dufour S, Lebon V, Befroy D, Cline GW, Enocksson S, Inzucchi SE, Shulman GI, Petersen KF. The effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes. Diabetes. 2002; 51:797–802. [PubMed: 11872682]

Medina-Gomez G, Gray SL, Yetukuri L, Shimomura K, Virtue S, Campbell M, Curtis RK, Jimenez-Linan M, Blount M, Yeo GS, Lopez M, Seppanen-Laakso T, Ashcroft FM, Oresic M, Vidal-Puig A. PPAR gamma 2 prevents lipotoxicity by controlling adipose tissue expandability and peripheral lipid metabolism. PLoS genetics. 2007; 3:e64. [PubMed: 17465682]

Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, Sponseller CA, Hampton K, Bacon BR. Interim results of a pilot study demonstrating the early effects of the PPAR-gamma ligand rosiglitazone on insulin sensitivity, aminotransferases, hepatic steatosis and body weight in patients with non-alcoholic steatohepatitis. J Hepatol. 2003; 38:434–440. [PubMed: 12663234]

Pettinelli P, Videla LA. Up-regulation of PPAR-gamma mRNA expression in the liver of obese patients: an additional reinforcing lipogenic mechanism to SREBP-1c induction. J Clin Endocrinol Metab. 2011; 96:1424–1430. [PubMed: 21325464]

Rahimian R, Masih-Khan E, Lo M, van Breemen C, McManus BM, Dube GP. Hepatic over-expression of peroxisome proliferator activated receptor gamma2 in the ob/ob mouse model of non-insulin dependent diabetes mellitus. Molecular and cellular biochemistry. 2001; 224:29–37. [PubMed: 11693197]

Reiberger T, Aberle JH, Kundi M, Kohrgruber N, Rieger A, Gangl A, Holzmann H, Peck-Radosavljevic M. IP-10 correlates with hepatitis C viral load, hepatic inflammation and fibrosis and predicts hepatitis C virus relapse or non-response in HIV-HCV coinfection. Antiviral therapy. 2008; 13:969–976. [PubMed: 19195322]

Sakakura Y, Shimano H, Sone H, Takahashi A, Inoue N, Toyoshima H, Suzuki S, Yamada N. Sterol regulatory element-binding proteins induce an entire pathway of cholesterol synthesis. Biochem Biophys Res Commun. 2001; 286:176–183. [PubMed: 11485325]

Schadinger SE, Bucher NL, Schreiber BM, Farmer SR. PPARgamma2 regulates lipogenesis and lipid accumulation in steatotic hepatocytes. American journal of physiology. Endocrinology and metabolism. 2005; 288:E1195–1205. [PubMed: 15644454]

Sermon F, Le Moine O, Gustot T, Quertinmont E, Louis H, Nagy N, Degraef C, Deviere J. Chronic alcohol exposure sensitizes mice to galactosamine-induced liver injury through enhanced keratinocyte chemoattractant and defective IL-10 production. J Hepatol. 2003; 39:68–76. [PubMed: 12821046]

Smith SA. Peroxisome proliferator-activated receptors and the regulation of mammalian lipid metabolism. Biochemical Society transactions. 2002; 30:1086–1090. [PubMed: 12440979]

Smith SJ, Cases S, Jensen DR, Chen HC, Sande E, Tow B, Sanan DA, Raber J, Eckel RH, Farese RV Jr. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. Nature genetics. 2000; 25:87–90. [PubMed: 10802663]

Staels B, Fruchart JC. Therapeutic roles of peroxisome proliferator-activated receptor agonists. Diabetes. 2005; 54:2460–2470. [PubMed: 16046315]

Sun X, Luo W, Tan X, Li Q, Zhao Y, Zhong W, Sun X, Brouwer C, Zhou Z. Increased plasma corticosterone contributes to the development of alcoholic fatty liver in mice. Am J Physiol Gastrointest Liver Physiol. 2013; 305:G849–861. [PubMed: 24113770]

Sun X, Tang Y, Tan X, Li Q, Zhong W, Sun X, Jia W, McClain CJ, Zhou Z. Activation of peroxisome proliferator-activated receptor-gamma by rosiglitazone improves lipid homeostasis at the adipose tissue-liver axis in ethanol-fed mice. Am J Physiol Gastrointest Liver Physiol. 2012; 302:G548–557. [PubMed: 22173916]

Tan X, Sun X, Li Q, Zhao Y, Zhong W, Sun X, Jia W, McClain CJ, Zhou Z. Leptin deficiency contributes to the pathogenesis of alcoholic fatty liver disease in mice. The American journal of pathology. 2012; 181:1279–1286. [PubMed: 22841822]

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome biology. 2002; 3 RESEARCH0034.

Villanueva CJ, Monetti M, Shih M, Zhou P, Watkins SM, Bhanot S, Farese RV Jr. Specific role for acyl CoA:Diacylglycerol acyltransferase 1 (Dgat1) in hepatic steatosis due to exogenous fatty acids. Hepatology. 2009; 50:434–442. [PubMed: 19472314]

Wang X, Kilgore MW. Signal cross-talk between estrogen receptor alpha and beta and the peroxisome proliferator-activated receptor gamma1 in MDA-MB-231 and MCF-7 breast cancer cells. Molecular and cellular endocrinology. 2002; 194:123–133. [PubMed: 12242035]

Way JM, Harrington WW, Brown KK, Gottschalk WK, Sundseth SS, Mansfield TA, Ramachandran RK, Willson TM, Kliewer SA. Comprehensive messenger ribonucleic acid profiling reveals that peroxisome proliferator-activated receptor gamma activation has coordinate effects on gene

expression in multiple insulin-sensitive tissues. Endocrinology. 2001; 142:1269–1277. [PubMed: 11181544]

Zhang W, Zhong W, Sun X, Sun Q, Tan X, Li Q, Sun X, Zhou Z. Visceral white adipose tissue is susceptible to alcohol-induced lipodystrophy in rats: role of acetaldehyde. Alcoholism, clinical and experimental research. 2015; 39:416–423.

Zhong W, Zhao Y, Sun X, Song Z, McClain CJ, Zhou Z. Dietary zinc deficiency exaggerates ethanol-induced liver injury in mice: involvement of intrahepatic and extrahepatic factors. PloS one. 2013; 8:e76522. [PubMed: 24155903]

Zhong W, Zhao Y, Tang Y, Wei X, Shi X, Sun W, Sun X, Yin X, Sun X, Kim S, McClain CJ, Zhang X, Zhou Z. Chronic alcohol exposure stimulates adipose tissue lipolysis in mice: role of reverse triglyceride transport in the pathogenesis of alcoholic steatosis. The American journal of pathology. 2012; 180:998–1007. [PubMed: 22234172]

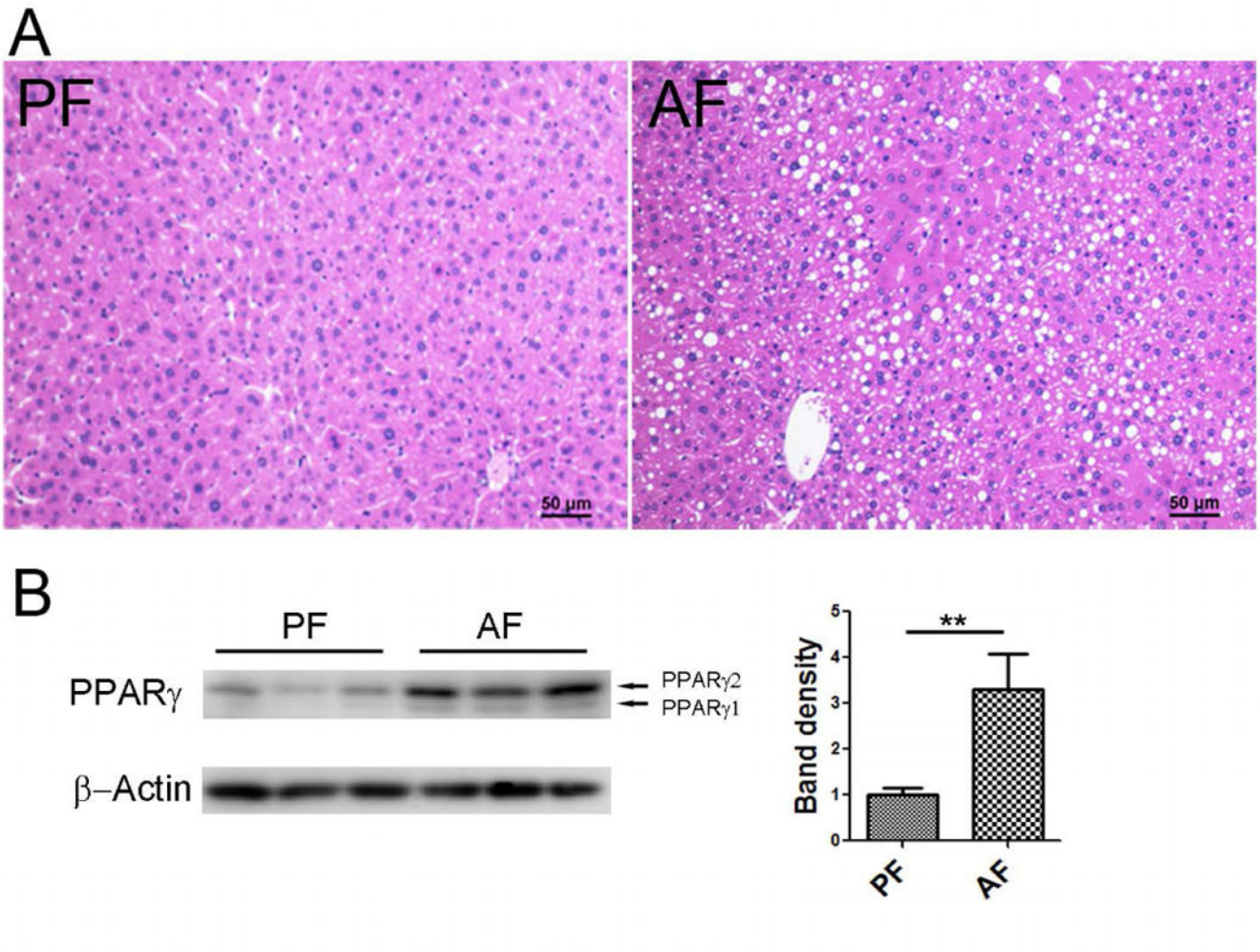

Figure 1.

Eight-week alcohol feeding induced steatosis and PPARγ in liver. WT mice were fed liquid diets containing alcohol (AF) or maltose dextrin (PF) for 8 weeks. A: liver histopathology (H-E staining). B: immunoblot analysis of hepatic PPARγ (left panel) and quantification of PPARγ levels by NIH ImageJ software (right panel) (Bethesda,MD). Data are expressed as means ± SD (n=3). $^{**}P < 0.01$.

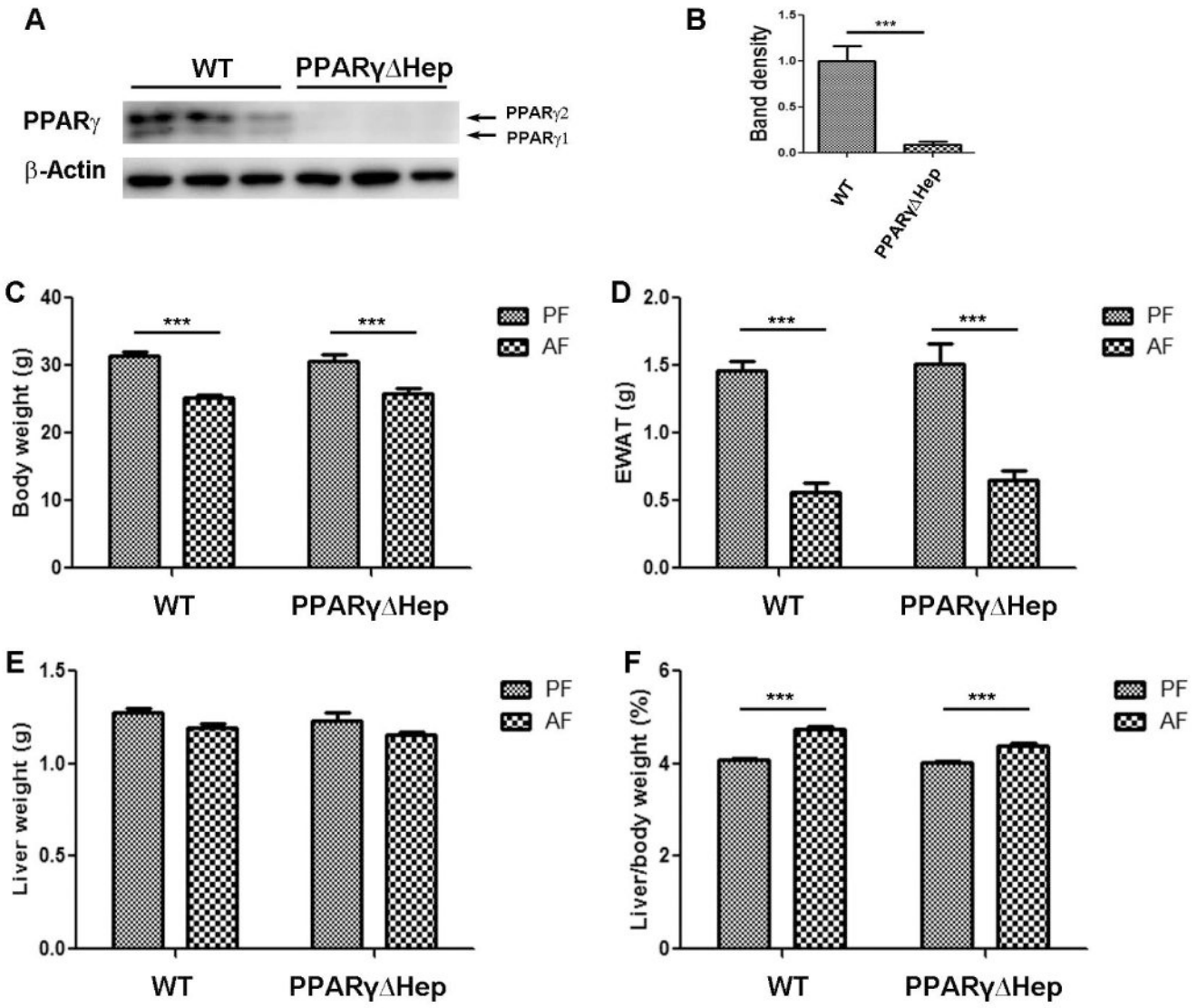

Figure 2.

Effects of alcohol feeding on body weight, EWAT weight, liver weight, and liver to body weight ratio. WT mice and PPARγΔHep mice were fed liquid diets containing alcohol (AF) or maltose dextrin (PF) for 8 weeks. EWATs and livers were taken. A: Immunoblot analysis of PPARγ. B. Quantification of band levels by NIH ImageJ software. C: Effects of alcohol feeding on body weight. D: Effects of alcohol feeding on EWAT weight. E: Effects of alcohol feeding on liver weight. F: Effects of alcohol feeding on liver to body weight ratio. The interaction between genotype and alcohol feeding is statistically significant (two-way ANOVA, $F_{(1,20)} = 11.04$, $P = 0.0034$). Data are expressed as means ± SD (n=3 in A, B, and n=6 in C-F). ***$P < 0.001$.

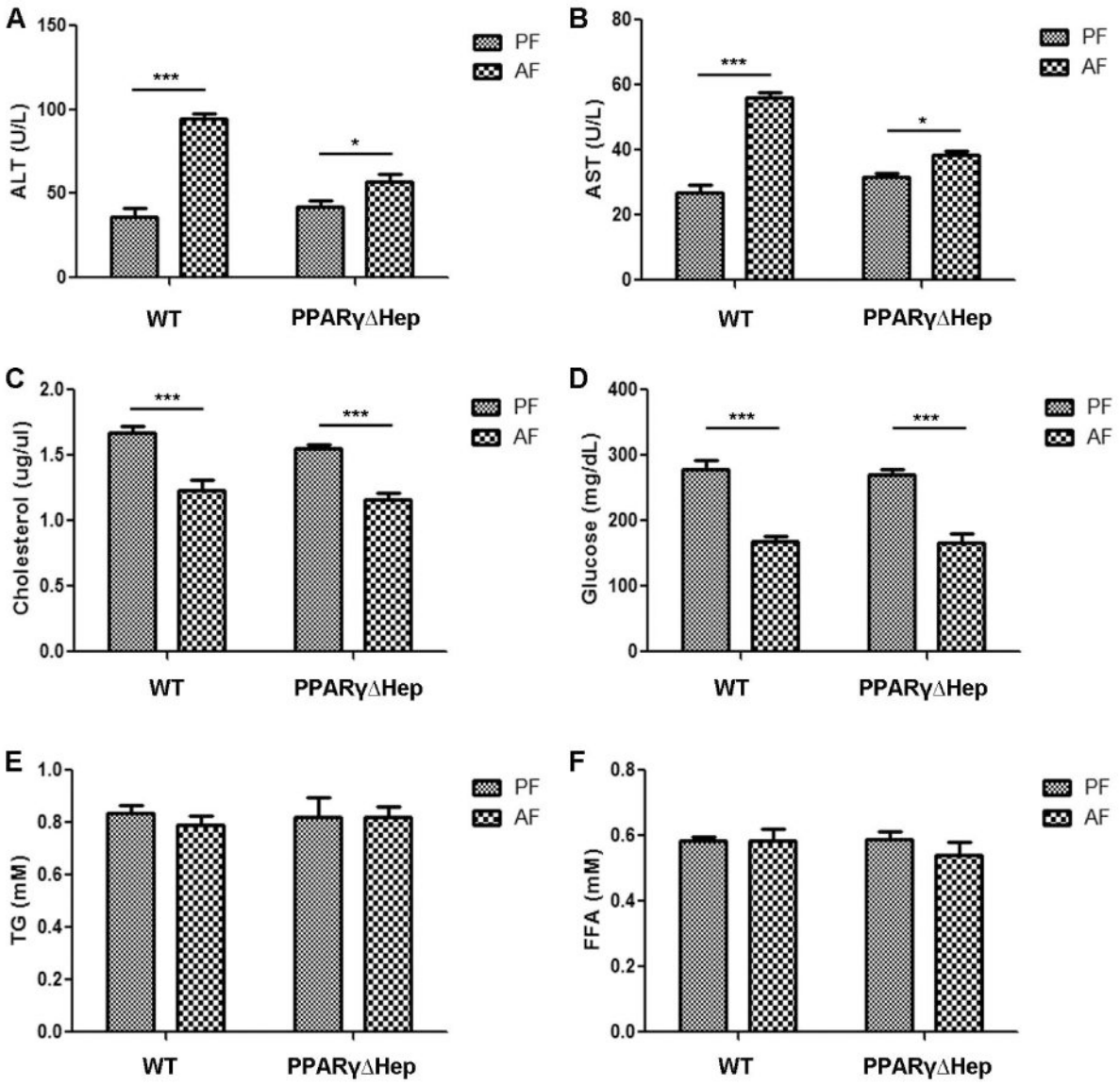

Figure 3.

Knockdown of PPARγ in hepatocytes attenuated alcohol-induced hepatic injury in mice. WT mice and PPARγΔHep mice were fed liquid diets containing alcohol (AF) or maltose dextrin (PF) for 8 weeks. A: Plasma ALT level. The interaction between genotype and alcohol feeding is statistically significant (two-way ANOVA, $F_{(1,28)} = 26.13$, $P < 0.0001$). B: Plasma AST level. The interaction between genotype and alcohol feeding is statistically significant (two-way ANOVA, $F_{(1,28)} = 41.10$, $P < 0.0001$).C: Plasma cholesterol content. D: Blood glucose concentration. E: Plasma TG content. F: Plasma FFA concentration. Data are expressed as means ± SD (n=8 in A, B, and F, n=5 in C and E, n=6 in D). *$P < 0.05$; ***$P < 0.001$.

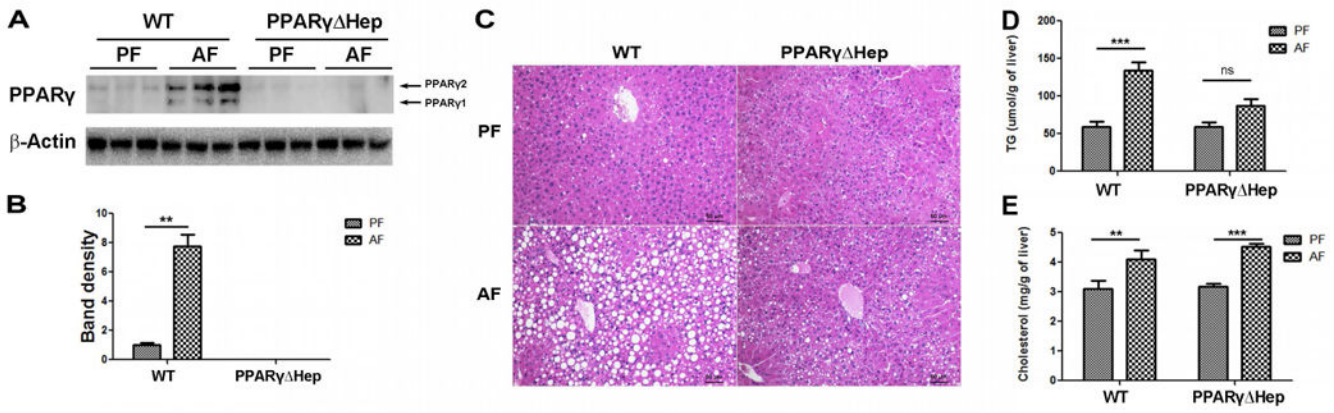

Figure 4.

Knockdown of PPARγ in hepatocytes attenuated alcohol-induced hepatic steatosis in mice. WT mice and PPARγΔHep mice were fed liquid diets containing alcohol (AF) or maltose dextrin (PF) for 8 weeks. A: Immunoblot analysis of PPARγ. B: Quantification of band levels by NIH ImageJ software. C: liver histopathology (H-E staining). D: Hepatic TG content. The interactions between genotype and alcohol feeding is statistically significant (two-way ANOVA, $F_{(1,16)} = 7.815$, $P = 0.0130$). E: Hepatic cholesterol content. Data are expressed as means ± SD (n=3 in A and B, n=5 in D, n=6 in E). $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.

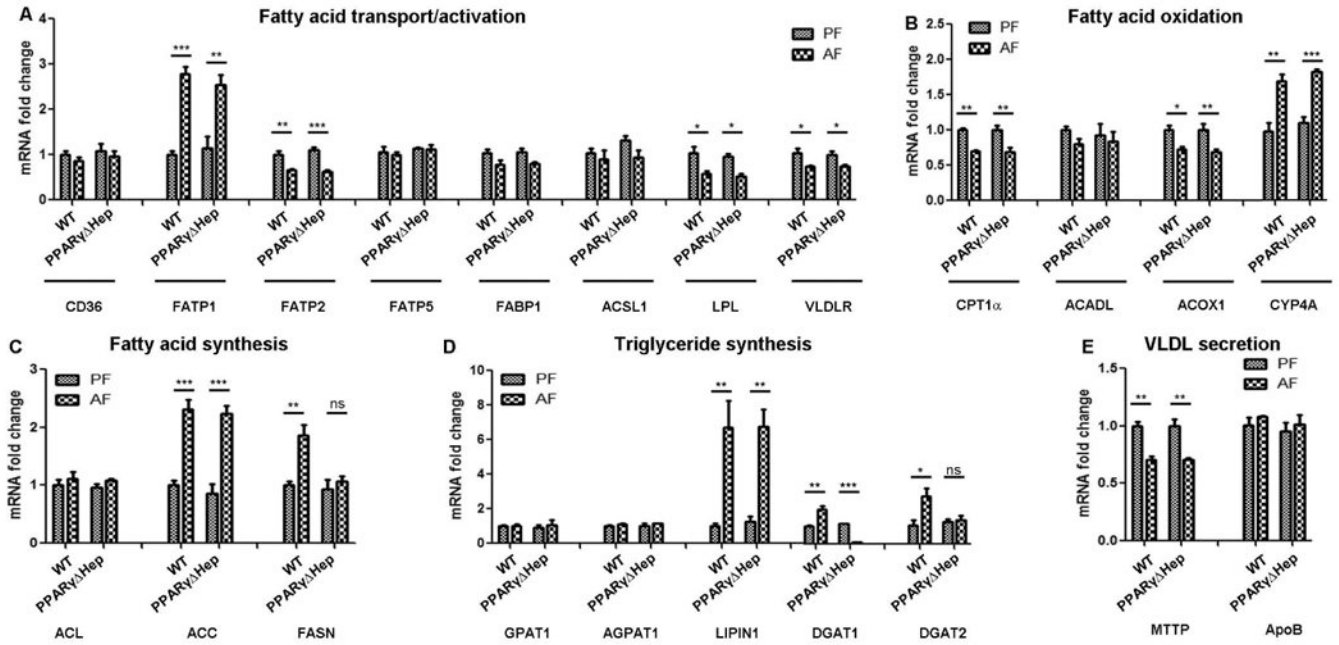

Figure 5.

Effect of hepatic PPARγ knockdown on the expression of genes involved in lipid metabolism. WT mice and PPARγΔHep mice were fed liquid diets containing alcohol (AF) or maltose dextrin (PF) for 8 weeks. A: The expression changes of genes related to fatty acid transport/activation. B: The expression changes of genes related to fatty acid oxidation. C: The expression changes of genes related to fatty acid synthesis. The interaction between genotype and alcohol feeding for FASN is statistically significant (two-way ANOVA, $F_{(1,8)} = 6.844$, $P = 0.0308$).D: The expression changes of genes related to TG synthesis. The interactions between genotype and alcohol feeding for DGAT1 and DGAT2 are statistically significant (two-way ANOVA, $F_{(1,8)} = 65.81$, $P < 0.0001$; $F_{(1,8)} = 5.356$, $P = 0.0494$).E: The expression changes of genes related to VLDL secretion. Data are expressed as means ± SD (n=3). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant.

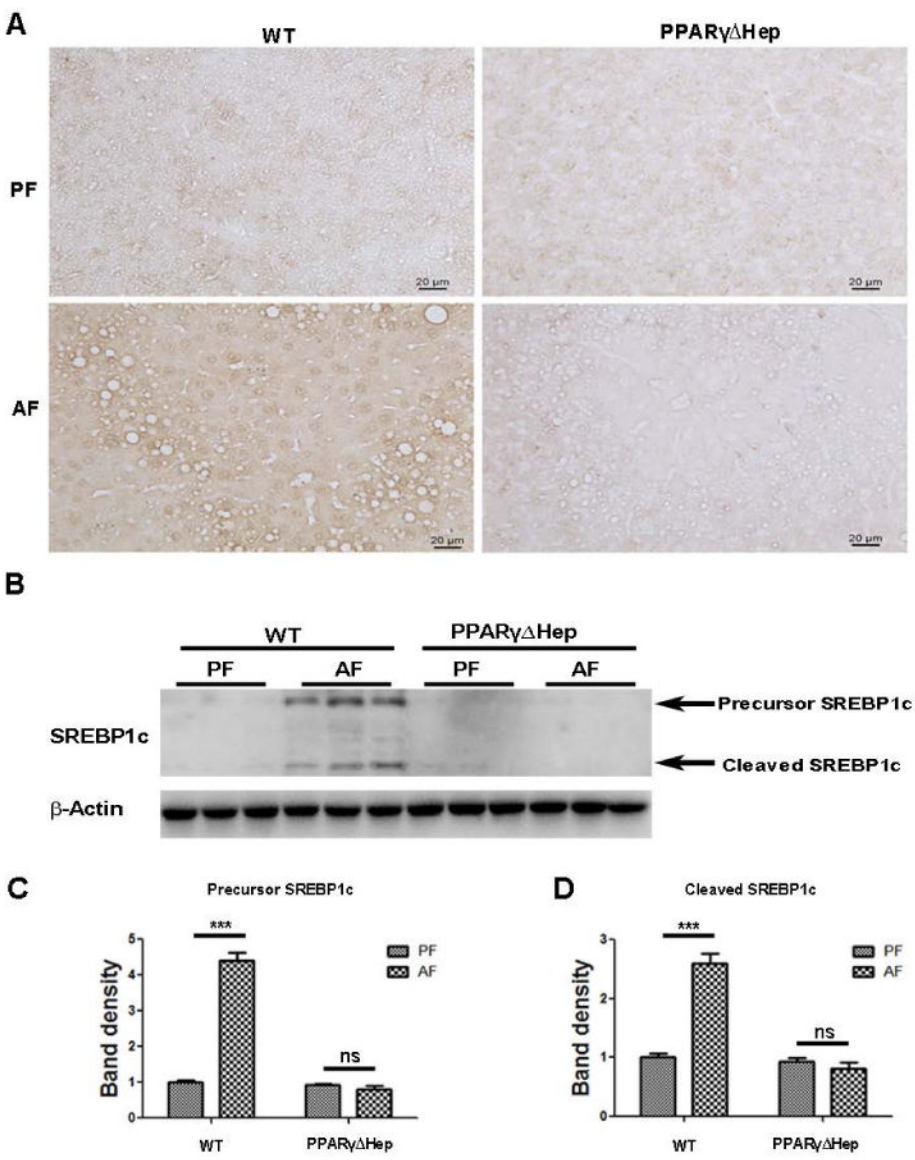

Figure 6.

Effect of hepatic PPARγ knockdown on alcohol-induced activation of SREBP-1c. A: Eight-week alcohol feeding significantly induced the nuclear translocation of SREBP-1c in WT mouse liver (lower left panel) which was abrogated by hepatic PPARγ knockdown (lower right panel). B: Hepatic PPARγ knockdown abolished alcohol-feeding-induced precursor SREBP-1c and cleaved SREBP-1c. C: Quantification of band levels of precursor SREBP-1c by NIH ImageJ software. D: Quantification of band levels of cleaved SREBP-1c by NIH ImageJ software. The interactions between genotype and alcohol feeding for precursor SREBP-1c and cleaved SREBP-1c are statistically significant (two-way ANOVA, $F_{(1,8)}$ = 182.0, $P < 0.0001$; $F_{(1,8)}$ = 62.40, $P < 0.0001$). Data are expressed as means ± SD (n=3). *** $P < 0.001$; ns, not significant.

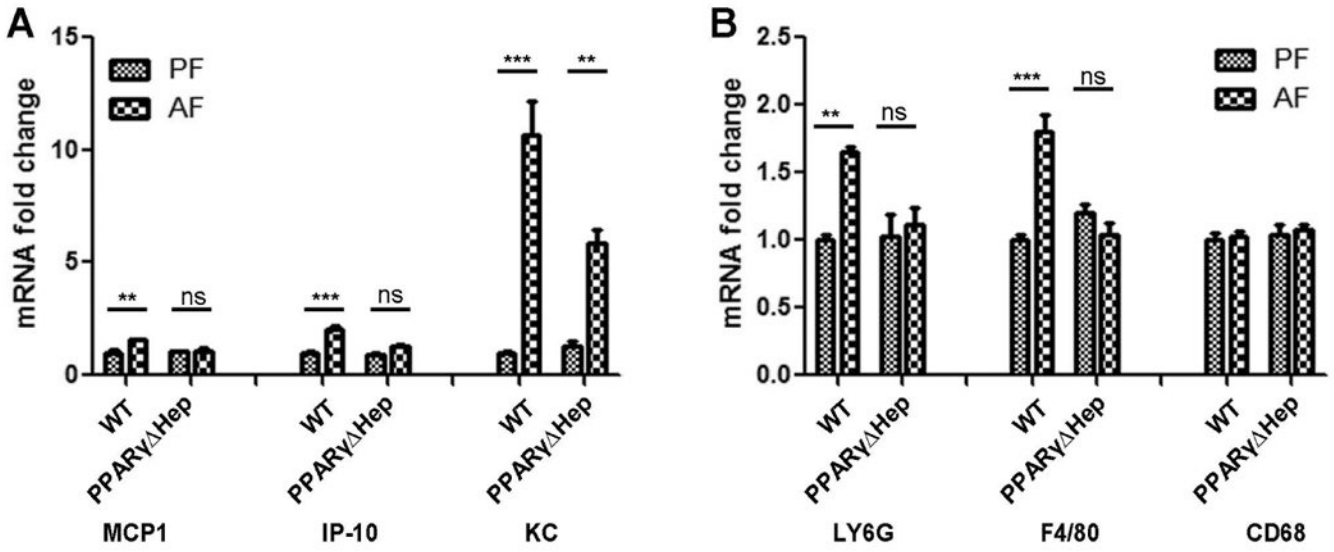

Figure 7.

Effect of hepatic PPARγ knockdown on inflammatory cell infiltration and inflammatory chemokine expression in the livers of mice fed alcohol for eight weeks. A: The expression changes of markers of inflammatory cell infiltration. The interactions between genotype and alcohol feeding for MCP1, IP-10, and KC are statistically significant (two-way ANOVA, $F_{(1,8)} = 9.137$, $P = 0.0165$; $F_{(1,8)} = 10.21$, $P = 0.0127$; $F_{(1,8)} = 9.290$, $P = 0.0159$).B: The expression changes of inflammatory chemokines. The interactions between genotype and alcohol feeding for LY6G and F4/80 are statistically significant (two-way ANOVA, $F_{(1,8)} = 7.256$, $P = 0.0273$; $F_{(1,8)} = 32.64$, $P = 0.0004$).Data are expressed as means ± SD (n=3). $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.

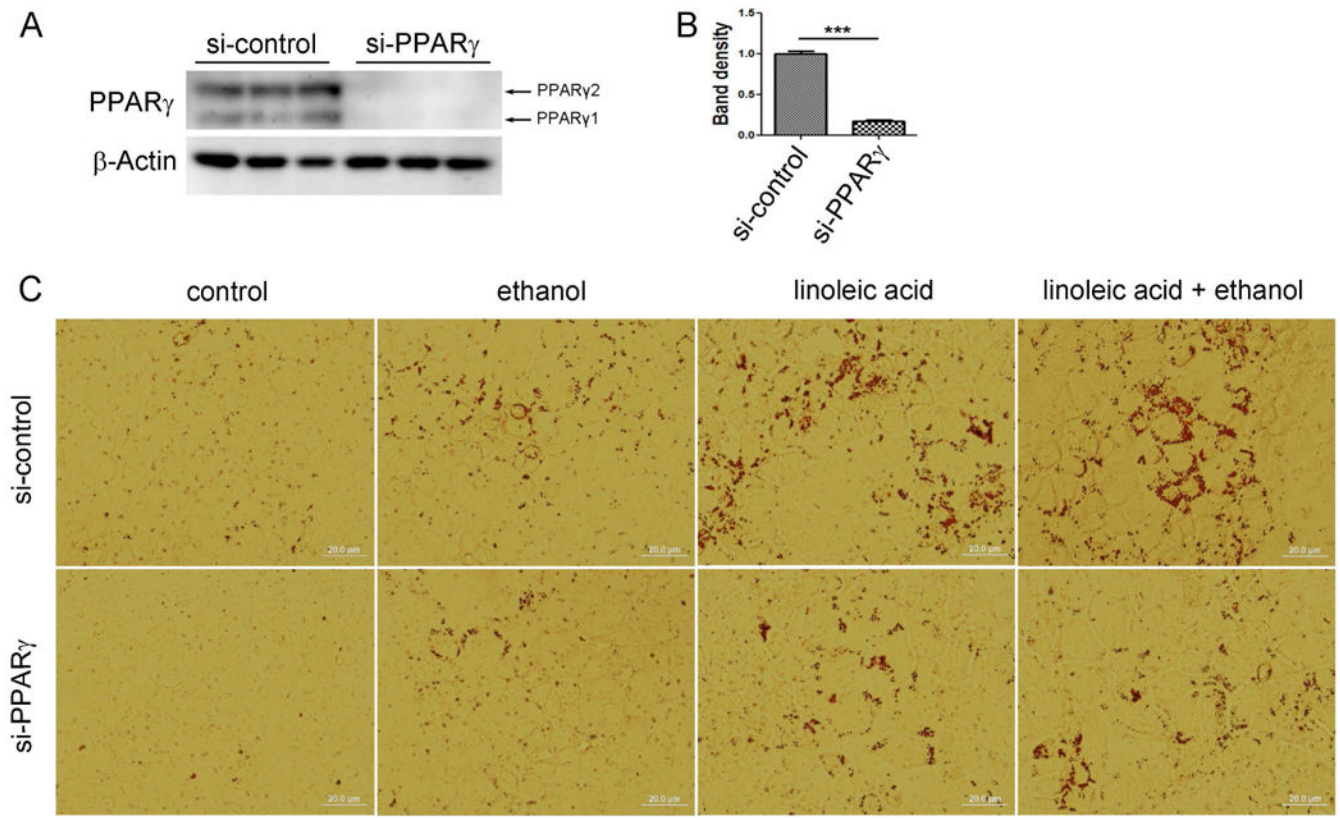

Figure 8.

Silencing of PPARγ inhibits alcohol-induced lipid accumulation *in vitro*. A: Immunoblot analysis of PPARγ. B. Quantification of band levels by NIH ImageJ software. C: Representative images for Oil red O staining of FL83B cells treated with ethanol, linoleic acid, or linoleic acid plus ethanol. Data are expressed as means ± SD (n=3).***$P < 0.001$

Table 1
Primer sequences used for qPCR analysis

Gene	Accession No.	Forward / Reverse (5′-3′)	Amplicon Size
CD36	NM_007643	ATGGGCTGTGATCGGAACTG/ GTCTTCCCAATAAGCATGTCTCC	110 bp
FATP1	NM_011977.3	TCCTAAGGCTGCCATTGTGG/ AGTCATAGAGCACATCGGCG	98 bp
FATP2	NM_011978	TCCTCCAAGATGTGCGGTACT/ TAGGTGAGCGTCTCGTCTCG	166 bp
FATP5	NM_009512	CTACGCTGGCTGCATATAGATG/ CCACAAAGGTCTCTGGAGGAT	103 bp
FABP1	NM_017399	ATGAACTTCTCCGGCAAGTACC/ CTGACACCCCCTTGATGTCC	118 bp
ACSL1	NM_007981	TGCCAGAGCTGATTGACATTC/GGCATACCAGAAGGTGGTGAG	101 bp
LPL	NM_008509.2	TTGCAGAGAGAGGACTCGGA/GGAGTTGCACCTGTATGCCT	125 bp
VLDLR	NM_013703.2	TGACGCAGACTGTTCAGACC/GGTTCGAGAAGGGCAGTTGA	190 bp
CPT1α	NM_013495	CTCCGCCTGAGCCATGAAG/CACCAGTGATGATGCCATTCT	100 bp
ACADL	NM_007381	TCTTTTCCTCGGAGCATGACA/GACCTCTCTACTCACTTCTCCAG	113 bp
ACOX1	NM_015729	TCCAGACTTCCAACATGAGGA/CTGGGCGTAGGTGCCAATTA	286 bp
CYP4A	NM_010011	TTCCCTGATGGACGCTCTTTA/GCAAACCTGGAAGGGTCAAAC	126 bp
ACL	NM_001199296	TTCCTCCTTAATGCCAGCGG/ TTGGGACTGAATCTTGGGGC	133 bp
ACC	NM_133360	CTTCCTGACAAACGAGTCTGG/CTGCCGAAACATCTCTGGGA	232 bp
FASN	NM_007988	GGAGGTGGTGATAGCCGGTAT/TGGGTAATCCATAGAGCCCAG	140 bp
GPAT1	NM_008149	ACAGTTGGCACAATAGACGTTT/CCTTCCATTTCAGTGTTGCAGA	139 bp
AGPAT1	NM_001163379	TAAGATGGCCTTCTACAACGGC/CCATACAGGTATTTGACGTGGAG	135 bp
LIPIN1	NM_172950	CATGCTTCGGAAAGTCCTTCA/GGTTATTCTTTGGCGTCAACCT	100 bp
DGAT1	NM_010046	TCCGTCCAGGGTGGTAGTG/TGAACAAAGAATCTTGCAGACGA	199 bp
DGAT2	NM_026384	GCGCTACTTCCGAGACTACTT/GGGCCTTATGCCAGGAAACT	172 bp
MTTP	NM_008642	CTCTTGGCAGTGCTTTTTCTCT/GAGCTTGTATAGCCGCTCATT	102 bp
ApoB	NM_009693	TTGGCAAACTGCATAGCATCC/TCAAATTGGGACTCTCCTTTAGC	142 bp
Ly6G	XM_001475753	CCACTCCTCTCTAGGACTTTCA/ACCTTGGAATACTGCCTCTTTC	117 bp
F4/80	NM_010130	TACCACTTGCCCAGCTTATG /GGGCCTTGAAAGTTGGTTTG	108 bp
CD68	NM_001291058	ATTGAGGAAGGAACTGGTGTAG/CCTCTGTTCCTTGGGCTATAAG	105 bp
MCP-1/Ccl2	NM_011333	GTCCCTGTCATGCTTCTGG/GCTCTCCAGCCTACTCATTG	125 bp
IP-10/Cxcl10	NM_021274	TCAGCACCATGAACCCAAG/CTATGGCCCTCATTCTCACTG	132 bp
KC/Cxcl1	NM_008176	AACCGAAGTCATAGCCACAC/CAGACGGTGCCATCAGAG	118 bp
18s rRNA	NR_003278	GTAACCCGTTGAACCCCATT/CCATCCAATCGGTAGTAGCG	151 bp