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Research Article

Low-Dose Alcohol Improves Lipid Metabolism through Store-Operated Ca^{2+} Channel-Induced PPARγ Expression in Obese Mice

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The relationship between low-dose alcohol consumption and lipid metabolism has been extensively studied during the last few decades. It has been reported that low-dose alcohol consumption upregulates the expression of peroxisome proliferator-activated receptor γ (PPARγ), a vital nuclear transcription factor involved in glucose and lipid metabolism. However, the possible molecular mechanism remains unclear. In the present study, the obese mouse model was established by HFD feeding for 12 weeks, and then alcohol was administered for 4 weeks. The results showed that low-dose alcohol consumption ameliorated HFD-induced glucose tolerance and insulin resistance in mice and decreased markedly the serum lipoprotein profiles levels and the size of lipid droplets that accumulated in the liver. Furthermore, low-dose alcohol consumption upregulated PPARγ and its target genes in obese mice and augmented the expression of relative proteins in store-operated Ca^{2+} channels (SOCs). Both ethylene glycol tetraacetic acid (EGTA), a Ca^{2+} chelator, and 2-aminoethoxydiphenyl borate (2-APB), a blocker of SOCs, abolished the alcohol-induced PPARγ upregulation. In conclusion, these results suggested that low-dose alcohol consumption could improve lipid metabolism through SOC-induced PPARγ expression in obese mice.

1. Introduction

Lipid metabolism disorder is widely recognized as a major cause of obesity, especially for people with obesity with a history of long-term excessive drinking [1]. Interestingly, low-dose alcohol consumption is beneficial to the regulation of metabolic homeostasis. Previous studies have reported that alcohol is involved in the regulation of lipid metabolism, and low-dose alcohol consumption improves the relevant indicators of lipid metabolism and reduces the incidence and mortality due to metabolic diseases [2–4]. This indicates that the amount of alcohol intake is a crucial factor in health outcomes. A question that arises is how much alcohol intake is appropriate? In a previous study, the daily intake of alcohol was within the range of 10–15 g for women and 20–30 g for men, which was considered to be low-dose drinking [5]. The relationship between alcohol intake and metabolic diseases showed a "J"-shaped curve; the beneficial effect of alcohol reached the maximum at 20 g per day and decreased to the minimum, or even to the level of damaging health, when the alcohol intake reached 72 g [5].

PPARγ is an important transcription factor involved in the regulation of glucose and lipid metabolism. Specific knockdown of PPARγ in hepatocytes, muscle, macrophages, or the brain could cause disorders of metabolism [6–9]. Alcohol is closely related to the activation of PPARγ transcription [10]. Studies have shown that low-dose alcohol consumption increases PPARγ mRNA levels and insulin sensitivity and

reduces the risk of diabetes in postmenopausal women [11]. The activation of PPARγ transcription is also regulated by intracellular Ca^{2+} [10]. In many cases, intracellular Ca^{2+}, acting as a "second messenger," carries a signal to one or more effector proteins, such as nuclear transcription factors. Under physiological conditions, variations of Ca^{2+} concentrations in different compartments—i.e., the extracellular space, the cytosol, and the endoplasmic reticulum (ER)—are employed to control a wide variety of activities within cells. A small amount of Ca^{2+} influx can cause obvious intracellular signal changes, which are a crucial link in regulating hepatocyte physiological activities, such as energy metabolism, bile acid metabolism, and protein synthesis [12–14].

Alcohol can promote intracellular Ca^{2+} influx via store-operated Ca^{2+} channels (SOCs), the main Ca^{2+} channels in the liver [13]. SOCs consist of two key structural proteins: the Orai calcium release-activated calcium modulator 1 (Orai1) and the Ca^{2+} sensor stromal interaction molecule 1 (STIM1) [15]. Orai1 proteins, which are transmembrane proteins, form Ca^{2+} channels in the cell membrane, conducting Ca^{2+} influx from the extracellular space. STIM1 proteins located in the ER membrane bind to Orai1 proteins, resulting in the opening of Orai1 channels [16, 17]. Bomfim et al. suggested a role for SOCs in regulating lipid metabolism and transcriptional reprogramming processes, and in participating in mitochondrial gene expression and fatty acid oxidation in cells or tissues [18]. Alcohol exposure increased the Ca^{2+} concentration in HepG2 cells, which were associated with SOCs [19]. However, it is unclear whether alcohol is involved in lipid metabolism through PPARγ activation induced by SOCs. We speculated that SOCs might be a key link in alcohol-induced PPARγ upregulation. To confirm this, we used EGTA to chelate extracellular Ca^{2+} in the cell medium and used 2-APB to block SOCs in L02 cells. The present study demonstrated that low-dose alcohol consumption increased PPARγ expression via SOCs, which might be a non-negligible mechanism by which alcohol regulates hepatic lipid metabolism in obese mice.

2. Materials and Methods

2.1. Reagents. Alcohol and thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Human insulin was purchased from Eli Lilly and Co. (Indianapolis, IN, USA). Blood lipid profile kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). EGTA was purchased from Solaribio (Beijing, China). 2-APB was purchased from Abcam (Cambridge, MA, UK). The PPARγ antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). STIM1 antibody and Orai1 antibody were purchased from Proteintech (Chicago, IL, USA). Activating transcription factor 4 (ATF4) antibody, activating transcription factor 6 (ATF6) antibody, X-box binding protein 1 (XBP-1) antibody, and C/EBP homologous protein (CHOP) antibody were purchased from cell signaling technology (Danvers, MA, USA). Adiponectin (APN) antibody, GAPDH antibody, and secondary antibody were purchased from AmyJet Scientific Inc. (Wuhan, China).

2.2. HFD-Induced Obese Mice and Alcohol Administration. All animal experiments met the requirements of experimental animal ethics. All experimental procedures were approved by the Ethics of Animal Experiments Committee of Xi'an Jiaotong University. Male C57BL/6 mice (6 weeks old) were randomly divided into a normal diet group (ND, $n = 8$), a high-fat diet group (HFD, $n = 8$), an alcohol intervention group (Alc, $n = 8$), and a high-fat diet group with alcohol intervention (HFD + Alc, $n = 8$). The mice in the ND and Alc groups were subjected to a standard diet. The HFD and HFD + Alc groups were fed a high-fat diet. After feeding for 12 weeks, the Alc and HFD + Alc groups were given 10% (v/v) alcohol daily at a dose of 0.8 g/kg via intragastric administration for 4 weeks. Meanwhile, the ND and HFD groups were intragastrically administered water.

2.3. Intraperitoneal Glucose Tolerance Test (IPGTT) and Intraperitoneal Insulin Tolerance Test (IPITT). The IPGTT and IPITT experimental methods were described in a previously published article [20]. For the IPGTT assay, the mice were fasted overnight for 12 hours at the end of the 16th week, and blood was collected from the tail. After measuring the basal blood glucose value, the mice were injected intraperitoneally with glucose solution (2.5 g/kg). Blood glucose levels were measured at 15, 30, 60, and 120 minutes after intraperitoneal injection of glucose. For the IPITT assay, after 4 hours of fasting, the mice were injected intraperitoneally with insulin (0.8 U/kg), and the basic blood glucose value was detected. Blood glucose levels were measured every 15, 30, 60, and 120 minutes. The IPGTT and IPITT of mice were evaluated by calculating the area under the curve (AUC) of the experimental result curve.

2.4. Detection of Serum APN Levels and Lipid Profile Analysis. At the end of the 16th week, serum samples were collected from all groups. APN, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels were detected according to the kit protocols.

2.5. Oil Red O Staining. To observe the lipid droplets of the liver, the tissues were processed into 6 μm cryosections, incubated with 10% formalin for 30 min, and then washed with running water for 1 min. The sections were stained with fresh Oil Red O Working Solution for 15 min. After washing with water, the sections were counterstained with hematoxylin dye for 1 min.

2.6. Cell Treatment and MTT Assay. L02, a normal human liver cell line, was used in the cell experiments. L02 cells were seeded into 6-well plates and cultured with DMEM. Cells were treated with 10 mM EGTA or 5 mM 2-APB for 1 h before exposure to 20 mM alcohol for 24 h.

The effect of alcohol on cell viability was evaluated by the MTT assay. L02 cells were seeded in 96-well plates and cultured until reaching 80% confluence. The L02 cells were treated with alcohol concentrations of 0 mM, 10 mM,

20 mM, 50 mM, 100 mM, and 200 mM for 24 h. Then, the L02 cells were treated with 5 mg/mL MTT for 4 h. Then, the DMEM was removed from all wells and washed with PBS solution 3 times. The formazan, the reaction product in L02 cells, was dissolved in 150 μL dimethyl sulfoxide (DMSO). The optical density of the DMSO solution was measured at a wavelength of 490 nm using a microplate reader.

2.7. Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR). RNA was isolated from the liver tissue, and complementary DNA was synthesized with a cDNA synthesis kit (Promega, WI, USA). RT-qPCR was performed using a 7500 real-time PCR system for 40 cycles. GAPDH was used as an internal control. The amplification results were calculated as $2^{-\Delta\Delta Ct}$. All primer sequences used in this study are listed in Table 1.

2.8. Western Blotting. Protein samples were extracted from the liver tissue or the L02 cells using ice-cold lysis buffer (Roche, Mannheim, Germany). Protein samples were separated on 10% SDS-PAGE gels and transferred to polyvinylidene fluoride membranes at a current of 300 mA through a 2-hour transfer process. The membranes were incubated with 5% bovine serum albumin for 1 h. The target proteins on the membranes were recognized by primary antibodies against h/m-PPARγ, h/m-APN, h/m-FABP4, h-STIM, h-Orai1, h-ATF4, h-ATF6, h-XBP-1, h-CHOP, and h/m-GAPDH. The next day, the membranes were incubated with the appropriate secondary antibodies. The target bands on the membranes were visualized by the ECL chemiluminescent system. The bands were analyzed by ImageJ software to obtain the density values. Values were normalized to GAPDH.

2.9. Statistical Analysis. SPSS 26.0 was used for data statistics, and the diagrams were made in GraphPad Prism 5. Quantitative data were expressed as mean ± SEM. Student's *t*-test or one-way ANOVA was used for comparison between two groups or among multiple groups. $P < 0.05$ was considered to be statistically significant.

3. Result

3.1. Low-Dose Alcohol Consumption Reduced Weight Gain in HFD-Induced Obese Mice. There were significant differences in body weight between the mice on different diets in the first 12 weeks, and HFD-induced obese mice gained weight more rapidly than the ND group. Compared with the ND group, the alcohol did not affect the weight of the mice in Alc group. After 4 weeks of alcohol intake in the HFD + Alc group (0.8 g/kg), the body weights were significantly different between the HFD group and the HFD + Alc group ($P < 0.05$) (Figure 1(a)). We also observed obvious changes in the visceral fat-to-body weight ratio ($P < 0.05$) (Figure 1(b)). These results indicated that low-dose alcohol intake reduced the body weight and visceral fat-to-body weight ratio in HFD-induced obese mice.

3.2. Low-Dose Alcohol Consumption Improved Glucose Tolerance and Insulin Sensitivity in HFD-Induced Obese Mice. Glucose or insulin tolerance was assessed to evaluate the effect of low-dose alcohol consumption on glucose metabolism. Blood glucose reached peak levels in the ND group and Alc group at 15 min and then decreased to normal levels within 120 min (Figure 2(a)). Compared with the ND group, blood glucose in the HFD group was higher at the peak level (26.7 mM) and did not return to normal levels within 120 min. However, the level of blood glucose was significantly improved in the HFD + Alc group and was much closer to normal levels after 120 min. The AUC results also showed similar trends ($P < 0.05$), indicating that low-dose alcohol consumption improved glucose metabolism in obese mice (Figure 2(b)). As shown in Figure 2(c), in the first 60 min, there was no remarkable difference between the ND group and HFD group in glucose levels after insulin injection, but blood glucose in the HFD group recovered quickly in the last 60 min, which indicated that mice had developed insulin resistance. The HFD + Alc group had lower glucose levels than the HFD group, which was in keeping with the AUC results ($P < 0.05$) (Figure 2(d)). The trends of blood glucose levels in the Alc group and ND group were basically consistent in the IPGTT and IPITT. Furthermore, the levels of serum APN in the HFD + Alc group were higher than those in the HFD group ($P < 0.05$), suggesting that low-dose alcohol might restore insulin sensitivity through the upregulation of serum APN in obese mice (Figure 2(e)).

3.3. Low-Dose Alcohol Consumption Attenuated Lipid Accumulation in Obese Mice. Serum lipoprotein profiles are important indicators of hepatic lipid metabolism. The present results showed that the serum levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) in the HFD group mice significantly increased by 54.9% and 707.7%, respectively (Figure 3(a)), and that the serum triglyceride (TG) level in the HFD group mice significantly increased by 131.0% compared with that of the ND group mice ($P < 0.05$) (Figure 3(b)). However, there was no difference in high-density lipoprotein cholesterol (HDL-C) levels among the groups ($P > 0.05$). Compared with the levels in the HFD group, the levels of TC, LDL-C, and TG in the HFD + Alc group decreased by 29.5%, 36.7%, and 46.3%, respectively ($P < 0.05$). There was no significant difference in TC, LDL-C, HDL-C, and TG levels between the Alc group and the ND group. The Oil Red O staining results showed hepatic steatosis (Figure 3(c)). Increased deposition of lipid droplets was seen in the liver of the HFD group mice. Alcohol administration did not completely improve excessive lipid accumulation in the HFD + Alc group but decreased the size of the lipid droplets compared with that of the HFD group. From the abovementioned results, low-dose alcohol consumption significantly reduced lipid accumulation in the livers of HFD-induced obese mice.

Table 1: Primers used for RT-qPCR.

Genes	Forward primer 5′–3′	Reverse primer 5′–3′
h-PPARγ	TCATGGCAATTGAATGTCGT	CCAACAGCTTCTCCTTCTCG
m-PPARγ	GTGATGGAAGACCACTCGCA	ACAGACTCGGCACTCAATGG
h-APN	TATGATGGCTCCACTGGTA	GAGCATAGCCTTGTCCTTCT
m-APN	TCAGTGGATCTGACGACACC	TGCCATCCAACCTGCACAA
h-FABP4	ACTGGGCCAGGAATTTGACG	CTCGTGGAAGTGACGCCTT
m-FABP4	ATGCCTTTGTGGGAACCTGG	GCTCTTCACCTTCCTGTCGT
h-STIM1	CCGCAGGGGTGTAGTAATCTG	AGCCAAAGGTCAAGTGCTCC
h-Orai1	CACTGGATCGGCCAGAGTTAC	GGACTCCTTGACCGAGTTGAG
h/m-GAPDH	TCCACCACCCTGTTGCTGTA	ACCACAGTCCATGCCATCAC

h: human; m: mouse. The primers were synthesized by Xi'an tsingke Ltd. (Xi'an, China).

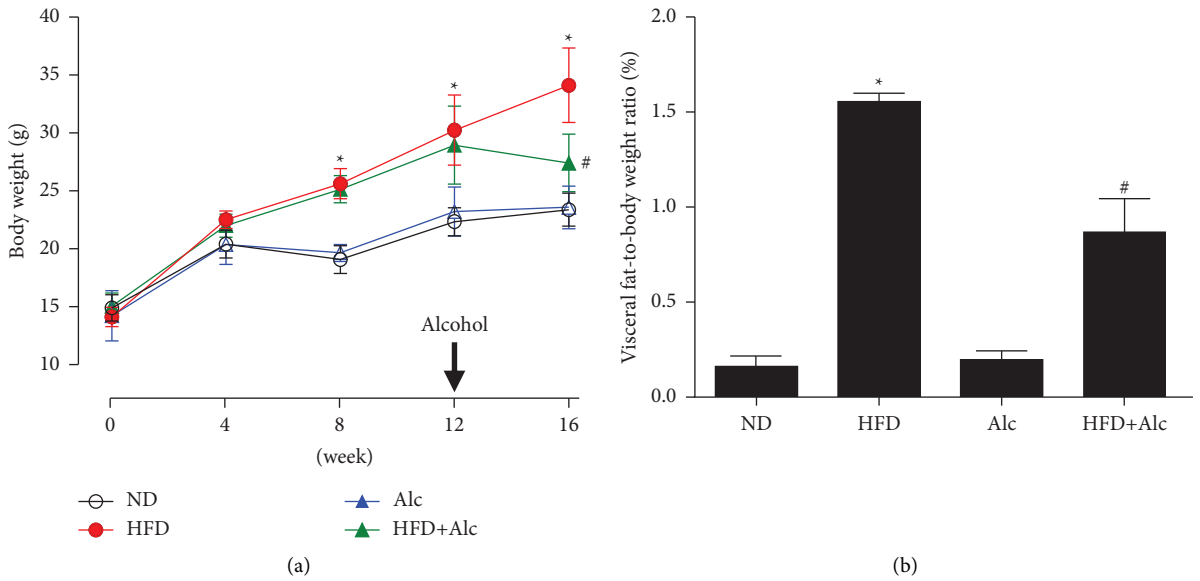

Figure 1: Low-dose alcohol consumption reduced weight gain in HFD-induced obese mice. Mice fed with ND or HFD for 12 weeks were administrated with alcohol at a dose of 0.8 g/kg by gavage for 4 weeks: (a) the change of body weight in all group mice and (b) visceral fat-to-body weight ratio was measured after 16 weeks. $n = 8$ per group. $^{*}P < 0.05$ vs. ND group; $^{\#}P < 0.05$ vs. HFD group.

3.4. Low-Dose Alcohol Consumption Increased PPARγ Expression in HFD-Induced Obese Mice and in L02 Cells. To understand the regulation of low-dose alcohol on PPARγ in HFD-induced obese mice, the expression levels of PPARγ and its target genes, APN, and FABP4 were analyzed at the protein and mRNA levels. Low-dose alcohol consumption significantly reversed PPARγ, APN, and FABP4 protein levels as well as reversing the mRNA levels that had been inhibited by obesity in the livers of HFD-induced mice ($P < 0.05$) (Figures 4(a)–4(c)). To further verify the effect of alcohol in vitro, we determined cell viability by MTT assay. As shown in Figure 4(d), L02 cells, which are normal human liver cells, were treated with different concentrations of alcohol for 24 h. The results showed that there was no difference in cell viability in the range from 0 mM to 50 mM alcohol; however, cell viability decreased by 12.2% and 22.6% at 100 mM and 200 mM alcohol, respectively ($P < 0.05$). Based on these results, L02 cells were then treated with 0 mM, 10 mM, 20 mM, and 50 mM alcohol, and the protein and mRNA expression levels of PPARγ, APN, and FABP4 significantly increased in a dose-dependent manner ($P < 0.05$) (Figures 4(e)–4(g)). Altogether, alcohol upregulated PPARγ expression and transcriptional activity.

3.5. Effect of Low-Dose Alcohol on ER Stress in L02 Cells. Although low-dose alcohol consumption did not cause L02 cell death (Figure 4(d)), it was not clear whether alcohol concentrations ranging from 0 mM to 50 mM caused ER stress and cell damage. To clarify this, we used different concentrations of alcohol to treat L02 cells and analyzed the relative protein expression levels in three key molecular pathways of ER stress: PERK-eIF2α-ATF4-CHOP, ATF6-bZip-CHOP, and IRE-(XBP-1) [21]. The present results showed that alcohol upregulated the relative protein expression levels of ATF4, ATF6, XBP-1, and CHOP in L02 cells at a concentration of 50 mM ($P < 0.05$) (Figures 5(a) and 5(b)). Altogether, alcohol concentrations of 0 mM to 20 mM did not cause ER stress in L02 cells, but a higher dose did. Based on the abovementioned results, we treated L02 cells with 20 mM alcohol in the following experiment.

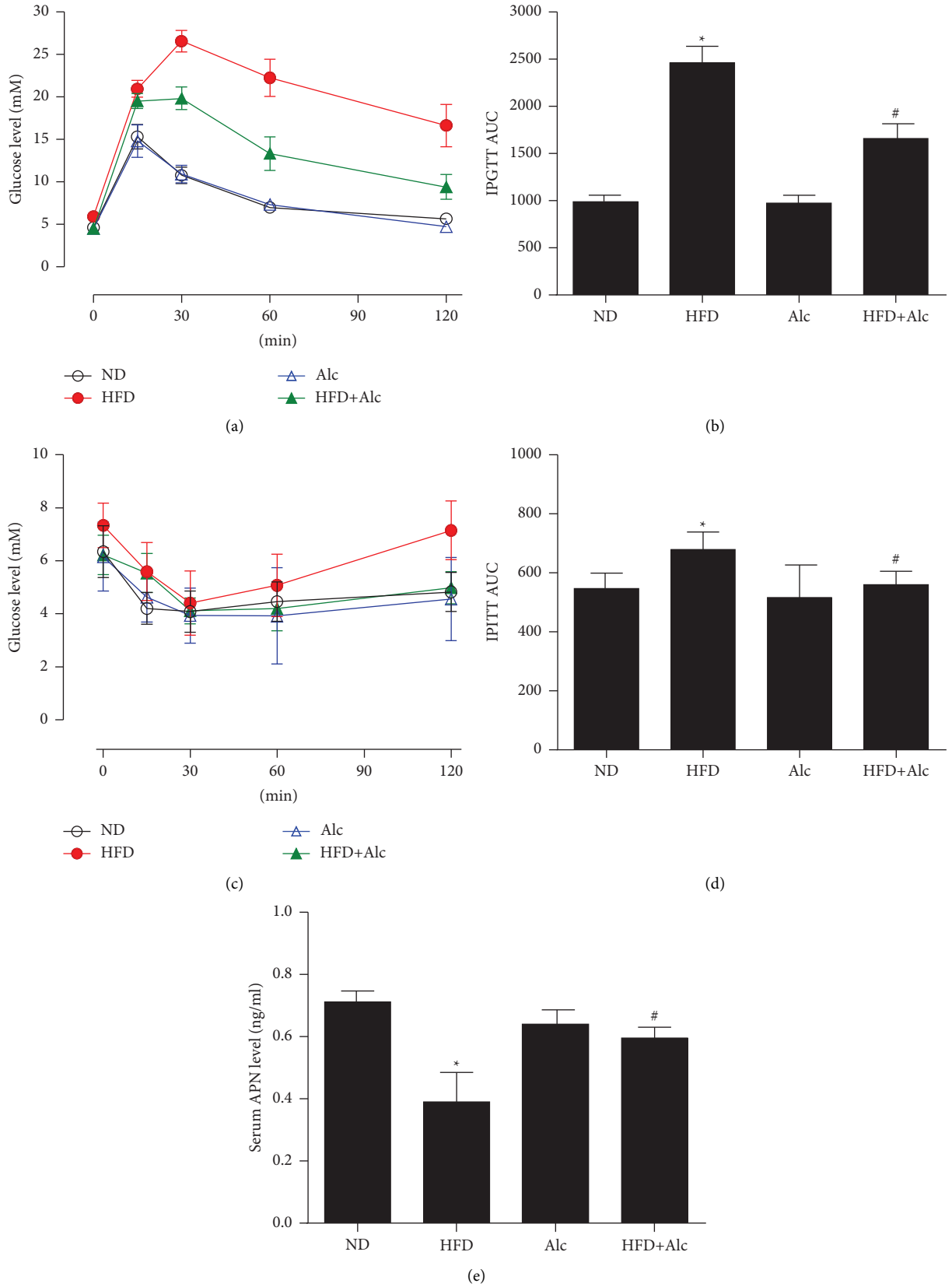

Figure 2: Low-dose alcohol consumption improved glucose tolerance and insulin sensitivity in obese mice. For IPGTT assay, mice were fasted for 12 h from 8 p.m. to 8 a.m. the next day. Mice were injected intraperitoneally with 2.5 g/kg glucose. (a) After baseline blood glucose levels were measured; blood glucose levels were measured at 15, 30, 60, and 120 min successively. (b) AUC was calculated according to IPGTT assay. For IPITT assay, the mice were injected intraperitoneally with 0.8 U/kg insulin. (c) After baseline blood glucose levels were measured, blood glucose levels were measured at 15, 30, 60, and 120 min successively. (d) AUC was calculated according to IPITT assay. (e) Serum APN levels were measured in every group at the end of last week. $n = 8$ per group. $^{*}P < 0.05$ vs. ND group; $^{\#}P < 0.05$ vs. HFD group.

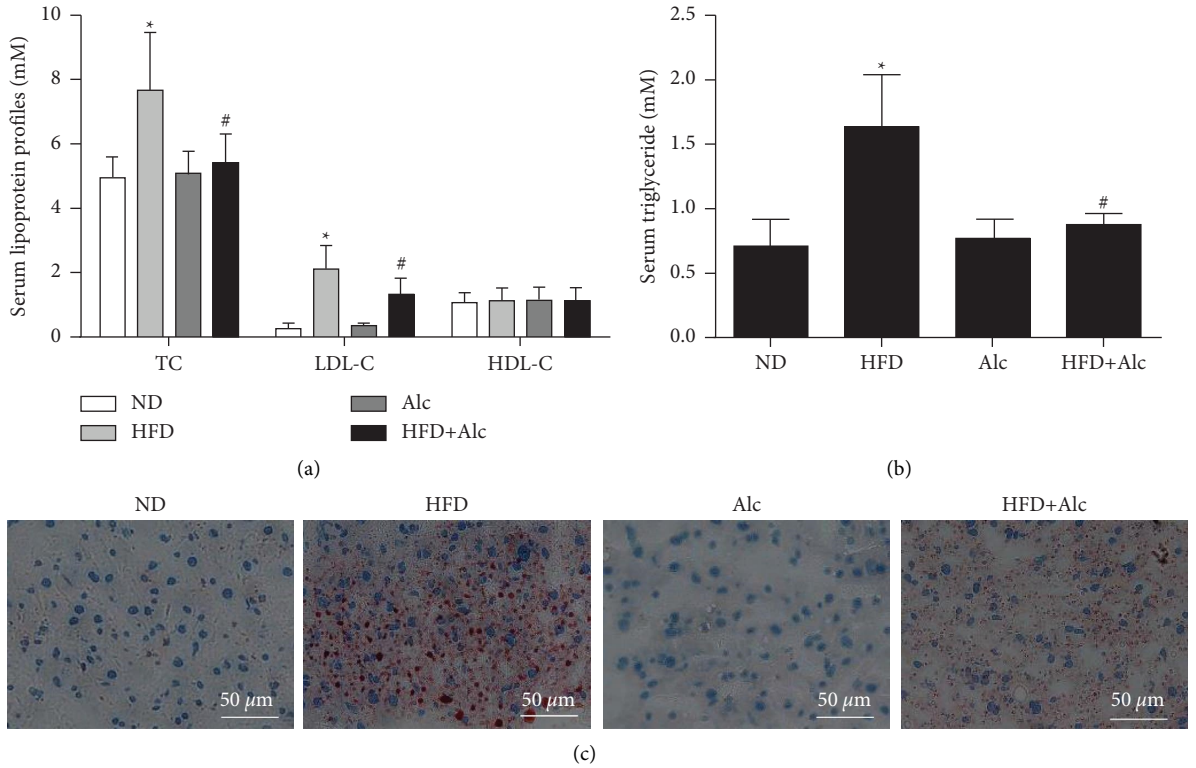

Figure 3: Low-dose alcohol attenuated lipid accumulation in HFD-induced obese mice. (a) Serum levels of lipoprotein profiles, $n = 6$ per group. (b) Serum TG levels, $n = 6$ per group. (c) Oil red O staining of livers in mice (40x). $^{*}P < 0.05$ vs. ND group; $^{\#}P < 0.05$ vs. HFD group.

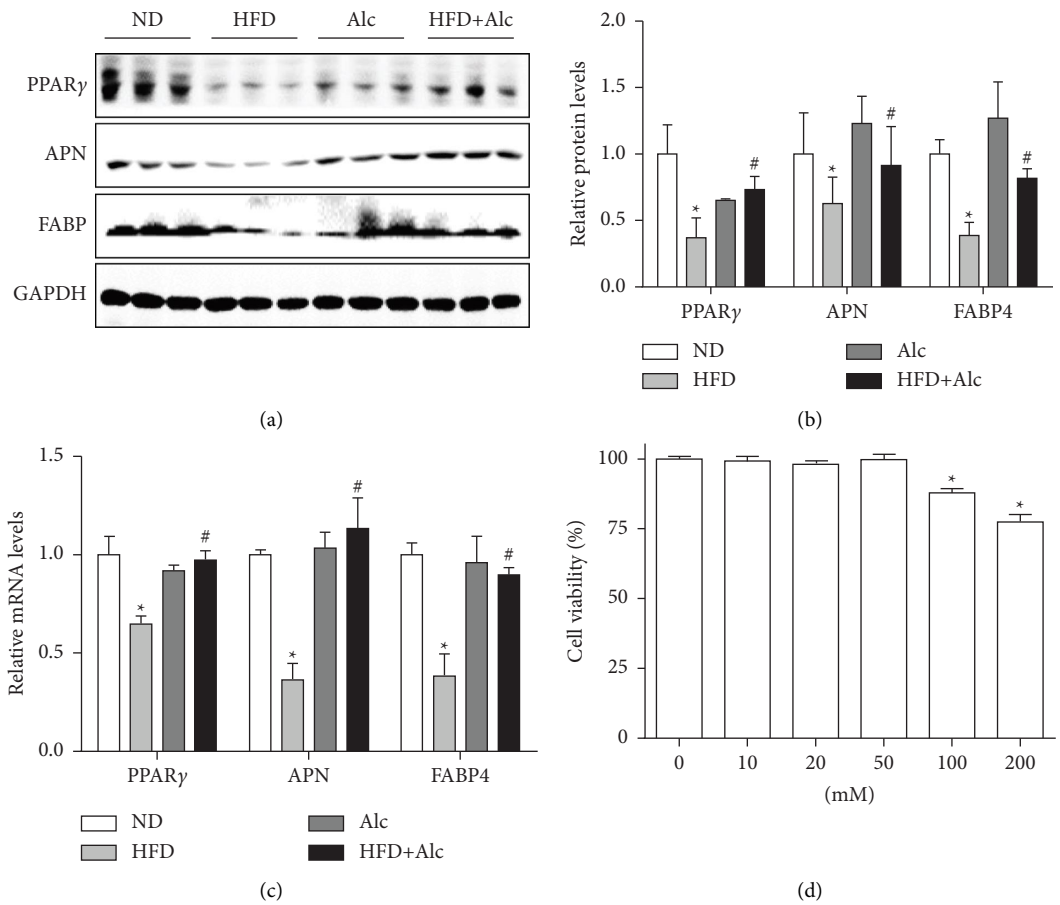

Figure 4: Continued.

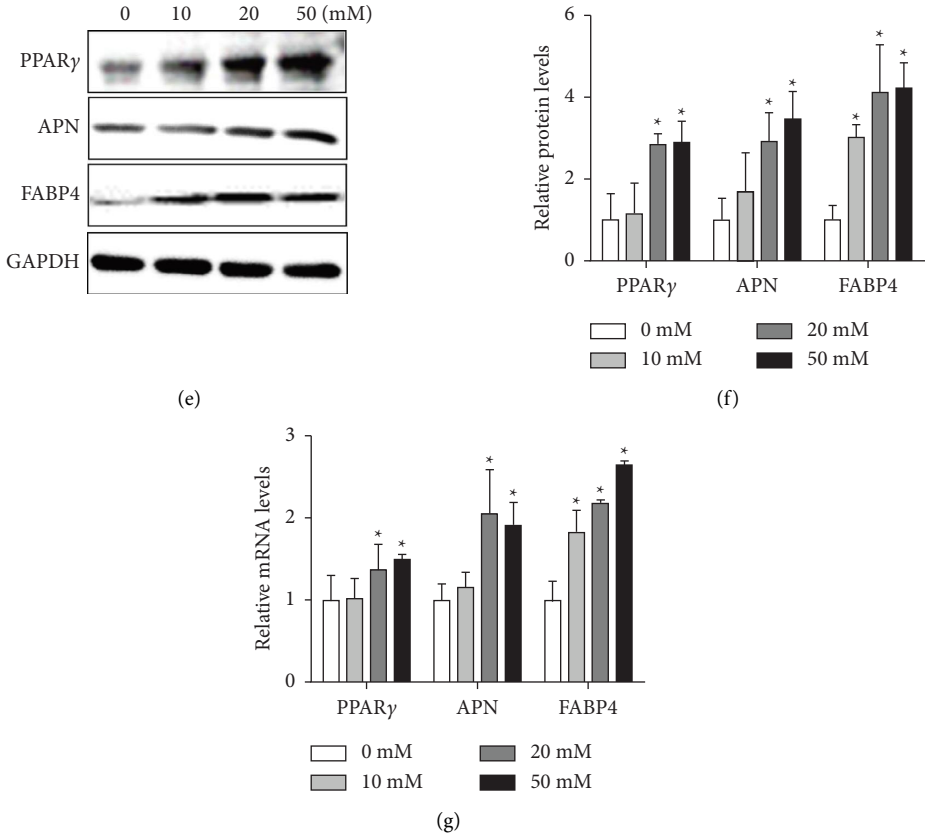

Figure 4: Low-dose alcohol consumption upregulated PPARγ expression. (a) Western blots of PPARγ, APN, and FABP4 in liver tissues of mice. (b) Quantification of PPARγ, APN, and FABP4 protein levels in liver tissues as in (a). (c) Hepatic mRNA levels of PPARγ, APN, and FABP4 were quantified by RT-qPCR. In MTT assay, L02 cells were treated with alcohol (0, 10, 20, 50, 100, and 200 mM) for 24 h. (d) Determination of cell viability by MTT in L02 cells ($n = 6$). L02 cells were treated with alcohol (0, 10, 20, and 50 mM) for 24 h. (e) Western blots of PPARγ, APN, and FABP4 in L02 cells. (f) Quantification of PPARγ, APN, and FABP4 protein levels in L02 cells as in (e). (g) mRNA levels of PPARγ, APN, and FABP4 were quantified by RT-qPCR in L02 cells. $n = 3$, $^{*}P < 0.05$ vs. ND group; $^{\#}P < 0.05$ vs. HFD group.

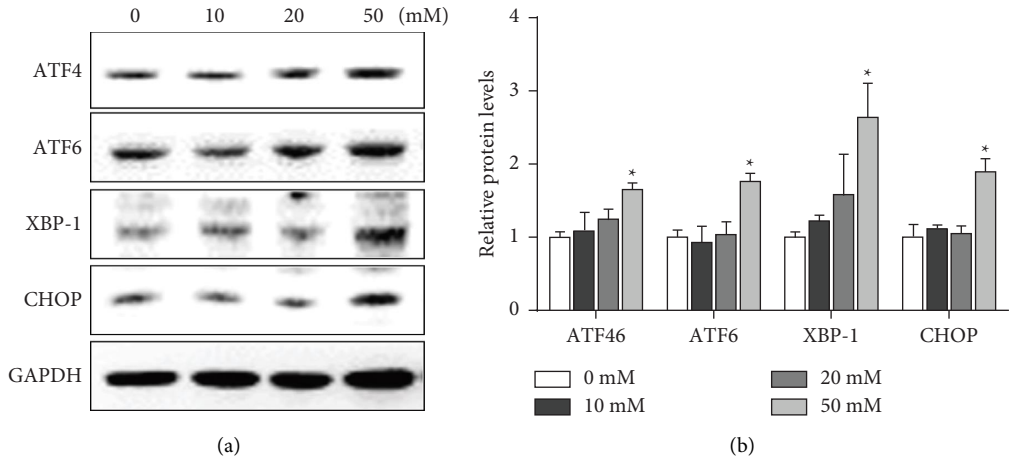

Figure 5: Effect of low-dose alcohol on ER stress in L02 cells. L02 cells were treated with 0 mM, 10 mM, 20 mM, and 50 mM alcohol. ATF4, ATF6, XBP-1, and CHOP protein levels were measured by western blotting. (a) Western blots of ATF4, ATF6, XBP-1, and CHOP. (b) Quantification of ATF4, ATF6, XBP-1, and CHOP protein levels as in (a). $n = 3$, $^{*}P < 0.05$ vs. 0 mM Alc.

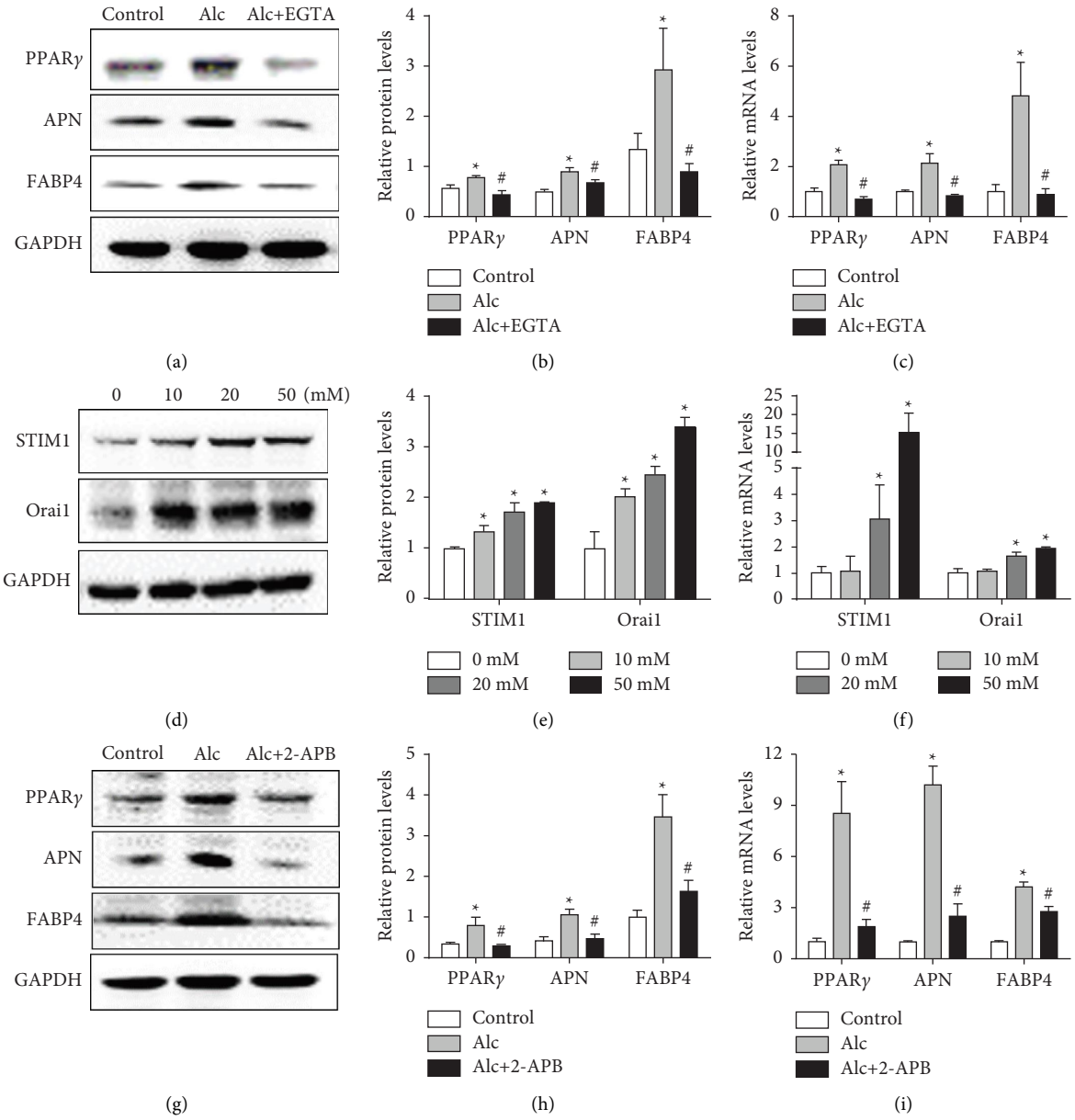

Figure 6: Low-dose alcohol consumption upregulated PPARγ by SOCs. L02 cells were pretreated with EGTA (10 mM) for 1 h and then treated with alcohol (20 mM) for 24 h. (a) Western blots of PPARγ, APN, and FABP4. (b) Quantification of PPARγ, APN, and FABP4 protein levels as in (a). (c) PPARγ, APN, and FABP4 mRNA levels were measured by RT-qPCR. L02 cells were treated with alcohol (0, 10, 20, and 50 mM) for 24 h. (d) Western blots of STIM1 and Orai1. (e) Quantification of STIM1 and Orai1 protein levels as in (d). (f) STIM1 and Orai1 mRNA levels were measured by RT-qPCR. L02 cells were pretreated with 2-APB (50 mM) for 1 h and then treated with alcohol (20 mM) for 24 h. (g) Western blots of PPARγ, APN, and FABP4. (h) Quantification of PPARγ, APN, and FABP4 protein levels as in (g). (i) PPARγ, APN, and FABP4 mRNA levels were measured by RT-qPCR. $n = 3$, $^{*}P < 0.05$ vs. control or 0 mM Alc; $^{\#}P < 0.05$ vs. Alc.

3.6. Low-Dose Alcohol Consumption Increased the Gene Expression of PPARγ through SOCs. To confirm whether PPARγ was regulated by SOCs, we used EGTA, a Ca^{2+} chelating agent, to chelate extracellular free Ca^{2+}. The results showed that the expression levels of PPARγ, APN, and FABP4 did not increase with respect to L02 cells treated with alcohol (Figures 6(a)–6(c)). This indicated that extracellular Ca^{2+} was involved in the regulation of PPARγ. To further clarify the molecular mechanism of alcohol action on PPARγ, we analyzed STIM1 and Orai1 in L02 cells treated with alcohol. The protein and mRNA levels of STIM1 and Orai1 increased in L02 cells ($P < 0.05$) (Figures 6(d)–6(f)). Meanwhile, 2-APB, a blocker of SOCs, inhibited the increase in alcohol-induced PPARγ expression ($P < 0.05$)

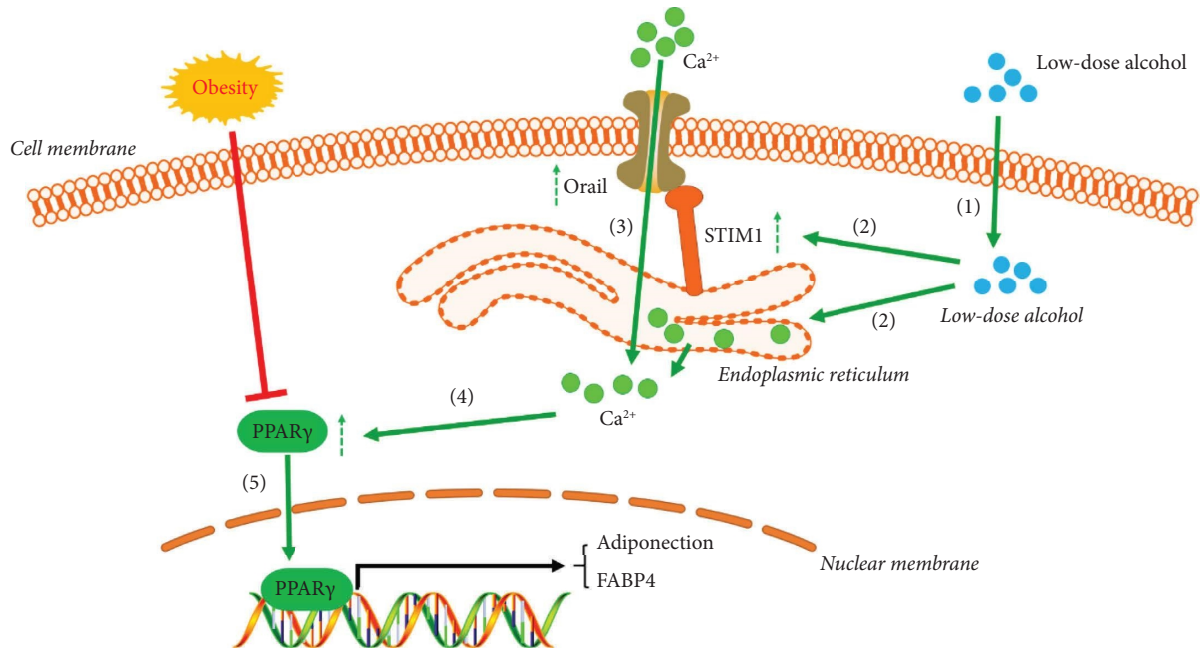

Figure 7: Molecular mechanism diagram illustrating low-dose alcohol consumption improved lipid metabolism through SOC-induced PPARγ expression in obese mice. (1) Low-dose alcohol entered the liver cells. (2) Low-dose alcohol upregulated the expressions of STIM1 and Orai1. (3) Extracellular Ca^{2+} entered cytoplasm through SOCs. (4) The expression of PPARγ was upregulated. (5) PPARγ entered the nucleus and regulated the target genes involved in glucose and lipid metabolism.

(Figures 6(g)–6(i)), indicating that alcohol could promote Ca^{2+} entry through SOCs to regulate PPARγ expression (Figure 7).

4. Discussion

Although recognized as an increasingly severe health problem, lipid metabolism disorders are still prevalent in humans. An increasing number of studies have reported that low-dose alcohol consumption increases PPARγ expression and insulin sensitivity and regulates glucose and lipid metabolism [22, 23]. However, it remains unclear how low-dose alcohol consumption improves metabolic disorders. Obesity is a predisposing factor for hepatic lipid metabolism disorder and causes insulin resistance [24]. We tested the effects of low-dose alcohol consumption on a well-established obese mouse model by HFD feeding. Compared with the HFD group, low-dose alcohol consumption reduced body weight and improved the impaired glucose tolerance and insulin resistance in the HFD + Alc group. Then, we measured serum TG and lipid profiles in all groups and observed the deposition of lipid droplets in the liver of the HFD group mice. Compared with the HFD group, alcohol intervention not only ameliorated the blood lipid levels but also reduced the size of liver lipid droplets. Taken together, low-dose alcohol consumption attenuated weight gain and improved glucose and lipid metabolism in HFD-induced obese mice.

PPARγ is a critical transcriptional regulator involved in glucose and lipid metabolism. It has been confirmed that a high-fat diet suppresses PPARγ expression, causing obesity and metabolic disorders [25, 26]. APN, a target gene of PPARγ, plays a beneficial role in energy homeostasis by increasing insulin sensitivity and improving glucose levels and lipid metabolism [27]. As another target gene in this study, FABP4 is known for its ability to bind free fatty acids. Yin et al. reported that FABP4 could be mediated by PPARγ stability and expression level [28]. In our study, low-dose alcohol improved TC, LDL, and TG levels in obese mice and upregulated the expression of PPARγ, APN, and FABP4 in the liver of obese mice as well as in L02 cells. One study showed that alcohol improved blood glucose and blood lipid levels, inhibited cholesterol synthesis, and changed weight by regulating key enzymes involved in lipid metabolism, such as hydroxymethylglutaryl coenzyme A reductase, paraoxonase-1 steroid regulatory element binding protein 2, and paraoxonase-1 [23]. Another study demonstrated that low-dose alcohol increased the acetylation levels of the PPARγ promoter region in liver cells [22]. The acetylation levels represent the transcriptional activity of key nuclear transcription factors in the genome [29], indicating that alcohol might affect PPARγ transcriptional activity. The transcriptional activation of PPARγ is regulated by many signaling molecules. As a previous study showed, Ca^{2+} was related to PPARγ expression [30]. To investigate the pathway by which alcohol regulates PPARγ, we pretreated L02 cells with EGTA to chelate extracellular Ca^{2+}. It was found that EGTA eliminated the increase in alcohol-induced PPARγ expression, suggesting that extracellular Ca^{2+} was involved in the regulation of PPARγ. In hepatocytes, SOCs are the main Ca^{2+} channel. It has been reported that alcohol upregulates the SOC key proteins STIM1 and Orai1 [31], and our experimental results also confirmed this finding. Consequently, we pretreated L02 cells with 2-APB, a blocker of

SOCs, and obtained the same result as treatment had been obtained with EGTA, which indicated that alcohol could upregulate PPARγ expression through SOCs in the liver.

Alcohol induces a change in the intracellular Ca^{2+} concentration in hepatocytes, which plays a physiological regulatory role. However, intracellular Ca^{2+} imbalance can cause damage to the ER, even inducing cell death. Previous studies have shown that heavy drinking or excessive alcohol exposure promotes Ca^{2+} influx in the liver, leading to Ca^{2+} overload [32]. Although Ca^{2+} overload could cause cell death, we observed that in the range of alcohol concentrations from 0 mM to 20 mM, low-dose alcohol-induced Ca^{2+} influx did not cause ER stress. This suggests that low-dose alcohol did not cause Ca^{2+} overload, and that ER stress effects were negligible within a certain range of alcohol concentrations. The alcohol application doses that cause L02 cell damage are usually more than or equal to 100 mM [33–35]; thus, our treatment dose (20 mM) was at a safe level. In some animal models, a dose of 5 g/kg/day alcohol has been used to induce liver injury [36, 37]. The alcohol intake of 0.8 g/kg/day in this study was a low dose. Although the definition of low-dose drinking varies from researcher to researcher, 20–30 g of alcohol a day for men might be considered a low-dose level [5], which was roughly comparable to the dose applied to animals in this study.

In conclusion, low-dose alcohol consumption regulated lipid metabolism in the livers of obese mice, and the main mechanism may be related to PPARγ expression induced by SOCs. The present study provides new insight into the treatment of lipid metabolism disorders and the rational use of alcohol.

Data Availability

The data that support the findings of this study are available from the corresponding author on request.

Additional Points

Practical Applications. Alcohol consumption is so closely related to human life that it has become indispensable to human beings. At present, numerous studies have confirmed that low-dose alcohol consumption has a regulatory effect on lipid metabolism of the body, but the specific mechanism remains unclear. The study confirmed that low-dose alcohol could increase the expression of PPARγ by SOCs to regulate the liver lipid metabolism. The research for our further understanding the biological effects of low doses of alcohol provides a theoretical basis. In addition, the result is a positive cue to "cut down on your drinking" for people who are unable to quit excessive drinking and suffer from metabolic disorders.

Ethical Approval

All animal experiments met the requirements of experimental animal ethics. All experimental procedures were approved by the Ethics of Animal Experiments Committee of Xi'an Jiaotong University.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Fan Li investigated the study, performed data curation, proposed the methodology, and wrote the original draft. Yanyan Zhu wrote the original draft and was responsible for resources. Huijuan Hu investigated the study and proposed the methodology. Jie Cheng investigated the study. Zhanqin Zhang reviewed and edited the manuscript. Xiaoming Sun was involved in project administration. Hao Hu conceptualized the study, supervised the study, and reviewed and edited the manuscript.

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