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Endoplasmic reticulum stress is involved in hepatic SREBP-1c activation and lipid accumulation in fructose-fed mice

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HIGHLIGHTS

- Fructose induces hepatic lipid accumulation and activates hepatic SREBP-1c.
- Fructose induces hepatic ER stress and decreases hepatic Insig-1 protein.
- PBA reduces fructose-induced hepatic ER stress and Insig1 depletion.
- PBA inhibits hepatic SREBP-1c activation and alleviates hepatic lipid accumulation.
- ER stress is involved in hepatic lipid accumulation in fructose-fed mice.

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ABSTRACT

A link between fructose drinking and nonalcoholic fatty liver disease (NAFLD) has been demonstrated in human and rodent animals. The aim of the present study was to investigate whether endoplasmic reticulum (ER) stress is mediated in the development of fructose-induced NAFLD. Female CD-1 mice were fed with 30% fructose solution for eight weeks. Hepatic lipid accumulation was assessed. Hepatic nuclear sterol regulatory element-binding protein (SREBP)-1c was measured. Results showed that hepatic SREBP-1c was activated in mice fed with fructose solution. Fatty acid synthase (fas) and acetyl-CoA carboxylase (acc), two target genes of SREBP-1c, were up-regulated. Fructose-evoked hepatic SREBP-1c activation seemed to be associated with insulin-induced gene (Insig)-1 depletion. An ER stress and unfolded protein response (UPR), as determined by an increased glucose-regulated protein (GRP78) expression and an increased eIF2α and PERK phosphorylation, were observed in liver of mice fed with fructose solution. Phenylbutyric acid (PBA), an ER chemical chaperone, not only significantly attenuated ER stress, but also alleviated fructose-induced hepatic Insig-1 depletion. PBA inhibited fructose-evoked hepatic SREBP-1c activation and the expression of SREBP-1c target genes, and protected against hepatic lipid accumulation. In conclusion, ER stress contributes, at least in part, to hepatic SREBP-1c activation and lipid accumulation in fructose-evoked NAFLD.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is gaining increasing recognition as a component of the epidemic of obesity in China (Fan and Farrell, 2009). Excessive accumulation of triglyceride (TG) in hepatocytes is the hallmark of NAFLD (Fabbrini et al., 2010). The spectrum of NAFLD ranges from simple fatty liver to a potentially progressive form, nonalcoholic steatohepatitis (NASH), which may lead to liver fibrosis and cirrhosis (Farrell and Larter, 2006). Although obesity, high fat diets and insulin resistance are recognized as risk factors for NAFLD (Anderson and Borlak, 2008; Fabbrini et al., 2010; Postic and Girard, 2008), other significant factors leading to NAFLD remain to be identified.

The consumption of soft drinks, which contain high concentrations of fructose, has markedly increased in the last three decades. Several studies have demonstrated that a diet rich in fructose might be an important risk factor in the development of NAFLD

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(Abdelmalek et al., 2010; Ouyang et al., 2008). A link between an increased consumption of fructose and the development of NAFLD has been demonstrated in the model of rodent animals (Nagai et al., 2009). According to several recent studies, an obvious hepatic lipid accumulation was observed in mice fed fructose solution (Bergheim et al., 2008; Spruss et al., 2009). Interestingly, pretreatment with antibiotics protected against hepatic lipid accumulation in mice fed with fructose solution (Bergheim et al., 2008). In addition, a significant increase in the level of endotoxin was observed in portal blood of mice fed with fructose solution, indicating that gut-derived endotoxin might play an important role in the development of fructose-induced NAFLD. Nevertheless, the molecular mechanisms of fructose-evoked NAFLD remain poorly understood.

An increasing evidence demonstrated that endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) might be involved in the development of NAFLD (Oyadomari et al., 2008; Rutkowski et al., 2008; Werstuck et al., 2001). According to a recent report, over-expression of glucose-regulated protein (GRP78), a molecular chaperone for protein folding in the ER, inhibited hepatic sterol regulatory element-binding protein (SREBP)-1c activation in obese mice (Kammoun et al., 2009). In addition, reversal of ER stress with chemical chaperones protected mice from obesity-induced NAFLD (Ozcan et al., 2006). The aim of the present study was to investigate whether ER stress is involved in hepatic SREBP-1c activation and lipid accumulation in fructose-fed mice.

2. Materials and methods

2.1. Reagents

Fructose and phenylbutyric acid (PBA) were from Sigma Chemical Co. (St. Louis, MO). SREBP-1, carbohydrate response element binding protein (ChREBP), X-box binding protein 1 (XBP-1), Liver X receptor alpha (LXRα), Lamin A/C, SCD-1, Insulin-induced gene 1 (INSIG-1), INSIG-2 and SCAP antibodies were from Santa Cruz Biotechnologies (Santa Cruz, CA). Glucose-regulated protein 78 (GRP78), phosphor-eIF2α and phosphor-PERK antibodies were from Cell Signaling Technology (Beverley, MA). β-actin antibody was from Boster Bio-Technology Co. Ltd (Wuhan, China). Chemiluminescence (ECL) detection kit from Pierce Biotechnology (Rockford, IL). TRI reagent was from Molecular Research Center, Inc (Cincinnati, OH). RNase-free DNase was from Promega Corporation (Madison, WI). Oil Red O was from Sigma Chemical Co. (St. Louis, MO). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO) if not otherwise stated.

2.2. Animals and treatments

Seventy-two female CD-1 mice (6–8 week-old; 20–22 g) were purchased from Beijing Vital River whose foundation colonies were all introduced from Charles River Laboratories, Inc. The animals were maintained on a 12-h light/dark cycle in a controlled temperature (20–25 °C) and humidity (50 ± 5%) environment for a period of 1 week before use. All mice were fed with regular diet. The food consumed consisted of standard rodent chow (50.76% carbohydrate, 15.44% fat, 33.8% protein calories), which were purchased from Jiangsu Cooperative Medical Biological Engineering Company Limited (Nanjing, China). To investigate the effects of fructose drinking on hepatic lipid accumulation, twenty-four mice were divided into two groups. Mice were free access to tap water containing 30% fructose or plain tap water for eight weeks. The concentration of fructose solution used in the present study referred to others (Spruss et al., 2009). To investigate the role of ER stress on fructose-induced hepatic SREBP-1c activation, forty-eight mice were divided into four groups. In fructose alone group, mice were free access to tap water containing 30% fructose for eight weeks. In control group, mice were free access to plain tap water for eight weeks. In fructose + PBA group, mice were free access to tap water containing 30% fructose for eight weeks and administered with PBA (100 mg/kg) for the final two weeks. In PBA alone group, mice were free access to plain tap water for eight weeks and administered with PBA (100 mg/kg) for the final two weeks. The dose of PBA used in the present study referred to others (Qi et al., 2004). Water and food intakes were assessed daily, whereas body weight was assessed weekly over the 8-week feeding period. All mice were sacrificed after feeding for eight weeks. Blood serum was collected for serum TG measurement. Liver was collected and either frozen immediately in liquid nitrogen for RT-PCR, Western blot and hepatic TG measurement, or fixed in neutral-buffered formalin for immunohistochemistry, or frozen-fixed in OCT mounting media for Oil Red O staining. All procedures on animals followed the guidelines for humane treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

2.3. TG assay

The level of serum TG was determined using a commercially available kit. For hepatic TG, liver samples were homogenized in ice-cold 2× phosphate-buffered saline (PBS). TG was extracted with methanol/chloroform (1:2), dried, and resuspended in 5% fat-free bovine serum albumin. The level of TG was determined using a commercially available kit. Hepatic TG was expressed as μmol/g liver.

2.4. Isolation of total RNA and real-time RT-PCR

Total RNA was extracted using TRI reagent. RNase-free DNase-treated total RNA (1.0 μg) was reverse-transcribed with AMV (Promega). Real-time RT-PCR was performed with a LightCycler® 480 SYBR Green I kit (Roche Diagnostics GmbH) using gene-specific primers as listed in Table 1. The amplification reactions were carried out on a LightCycler® 480 Instrument (Roche Diagnostics GmbH) with an initial hold step (95 °C for 5 min) and 50 cycles of a three-step PCR (95 °C for 15 s, 60 °C for 15 s, 72 °C for 30 s).

2.5. Immunoblots

Nuclear extracts were separated electrophoretically by SDS-PAGE and transferred to a polyvinylidene fluoride membrane. The membranes were incubated for 2 h with the following antibodies: SREBP-1, ChREBP, NF-κB p50 and NF-κB p65. Lamin A/C was used as a loading control for nuclear proteins. Hepatic total lysates were separated electrophoretically by SDS-PAGE and transferred to a polyvinylidene fluoride membrane. The membranes were incubated for 2 h with the following antibodies: SCD-1, iNOS, Insig-1, Insig-2, GRP78, phosphor-PERK and phosphor-eIF2α. β-Actin was used as a loading control for total proteins. After washes in DPBS containing 0.05% Tween-20 four times for 10 min each, the membranes were incubated with goat anti-rabbit IgG antibody for 2 h. The membranes were then washed for four times in DPBS containing 0.05% Tween-20 for 10 min each, followed by signal development using an ECL detection kit.

2.6. Oil Red O staining

To determine hepatic lipid accumulation, frozen sections of liver (10 μm) were stained with Oil Red O for 10 min, washed, and counterstained with hematoxylin for

Table 1
Oligonucleotide sequence of primers for real-time RT-PCR.

Gene	Forward (5′–3′)	Reverse (5′–3′)
Gapdh	ACCCCAGCAAGGACACTGAGCAAG	GGCCCCTCCTGTTATTATGGGGGT
Fas	CGCTCGGCTCGATGGCTCAG	CCAGCACCACGGCATGCTCA
Acc	CCGTTGGCCAAAACTCTGGAGCTAA	GAGCTGACGGAGGCTGGTGACA
Scd-1	CGGTCATCCCATCGCCTGCTCT	GTAGGCGAGTGGCGGAACTGC
Cd36	CACAGCTGCCTTCTGAAATGTGTGG	TTTCTACGTGGCCCGGTTCTAATTC
Srebp-1	GTGAGGCGGCTCTGGAACAGAC	ATAGGGGGCGTCAAACAGGCC
Lxr α	GGGGGTGACTGAGAAGCAGTCC	CATTGAGGCCTCCAGCCACAAGG
Grp78	CTGGCCGAGACAACACTGACCT	GCGACGACGGTTCTGGTCTCAC
Trib3	TCCCAGAGTCCGAGGCTGCC	AGGTCGCGCAAGACAAGCCC
Insig1	TCCCGCGAGGCATGATTGCG	CTGTGGTTCCCGCTCGCTGG
Insig2	TCTCCGGGCAGAGCTCAGGATT	TGGGCCACACTTTTTAGGCCGAG
Scap	AAACACCGGACCGCGGG	CAGCAGACATCATCTGCAGGCACC
Cpt1a	TTCCAGACGTCTCTGCCGCG	GGGCCAGTGCTGTCATGCGT
Cyp4a10	GATGGTTCTGGGGAAGCAAGGCC	AAGGCTGGGGTTAGCATCCTCCT
Cyp4a14	TGCTTACAGTGTCTCTCGGGGAGC	CCGCCGATGCTGGAACCACTT

45 s. Representative photomicrographs were captured at 400× magnification using a system incorporated in the microscope.

2.7. Statistical analysis

Quantified data were expressed as means ± SEM at each point. Homogeneity of variances was analyzed employing Levene's test. If variances were homogenous, ANOVA was used, followed by the Student–Newman–Keuls post hoc test were used to determine differences among different groups. Student *t*-test was used to determine differences between two groups. The Krusakal–Wallis test was used when variances were not homogenous. Differences between groups were analyzed using the Mann–Whitney *U*-test.

3. Results

3.1. Hepatic TG content and lipid accumulation

As shown in Table 2, average food intake was significantly decreased in fructose-fed mice. No significant difference on total caloric intake per day was observed between mice fed with fructose solution and those fed with water. The absolute and relative liver weights were significantly increased in mice fed with fructose solution. The level of serum and hepatic cholesterol was significantly increased in mice fed fructose solution (Table 2). As shown in Fig. 1A, an obvious hepatic lipid accumulation was observed in mice fed fructose solution. Correspondingly, the level of serum and hepatic TG was significantly increased in mice fed with fructose solution (Fig. 1B and C).

Table 2
Physiologic and serum parameters in control and mice fed with 30% fructose solution for eight weeks.

	Control (n = 12)	Fructose (n = 12)
Liquid intake (mL/day)	5.45 ± 0.27	5.47 ± 0.17
Chow intake (g/day)	4.09 ± 0.02	2.06 ± 0.07**
Total energy intake (kJ/day)	50.59 ± 0.24	51.24 ± 1.09
Body weight (g)	31.02 ± 0.30	32.91 ± 0.78**
Liver weight (g)	1.28 ± 0.03	1.61 ± 0.07**
Liver to body weight ratio (%)	4.12 ± 0.11	4.89 ± 0.16**
Hepatic total cholesterol (μmol/g liver)	9.59 ± 0.29	12.84 ± 0.62**
Serum total cholesterol (mmol/L)	1.90 ± 0.15	2.86 ± 0.25**
Serum HDL (mmol/L)	1.60 ± 0.10	2.24 ± 0.20**
Serum VLDL (mmol/L)	0.35 ± 0.02	0.52 ± 0.04**

Data are expressed as means ± SEM.
** $P < 0.01$ versus control group.

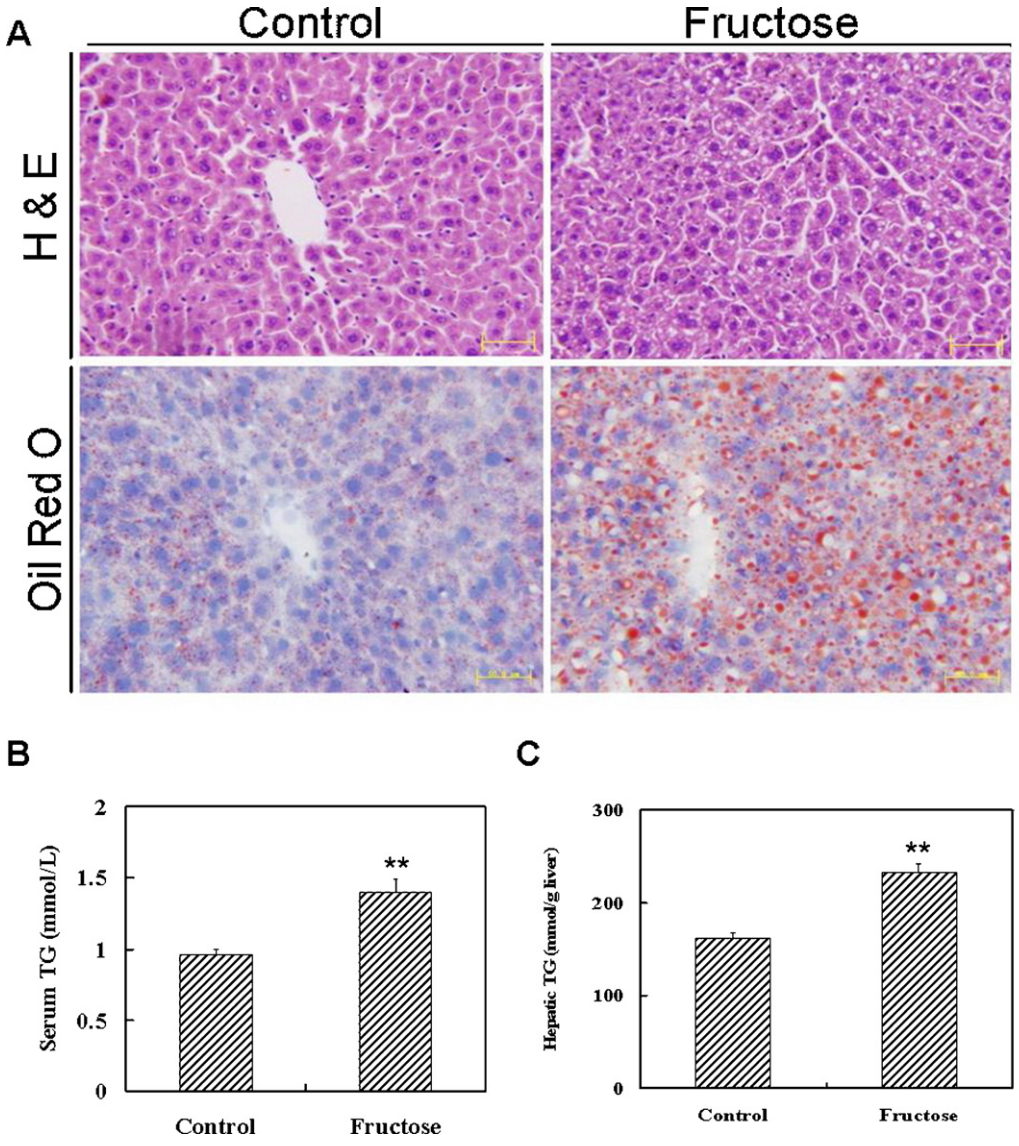

Fig. 1. Chronic fructose drinking induces hepatic lipid accumulation. (A) Liver histology and lipid accumulation. Liver sections were stained with either H&E (top row) or Oil Red O (bottom row). Original magnification, 400×. (B) Serum and (C) hepatic triglycerides (TG) contents. Data were expressed as means ± SEM (n = 12), **$P < 0.01$.

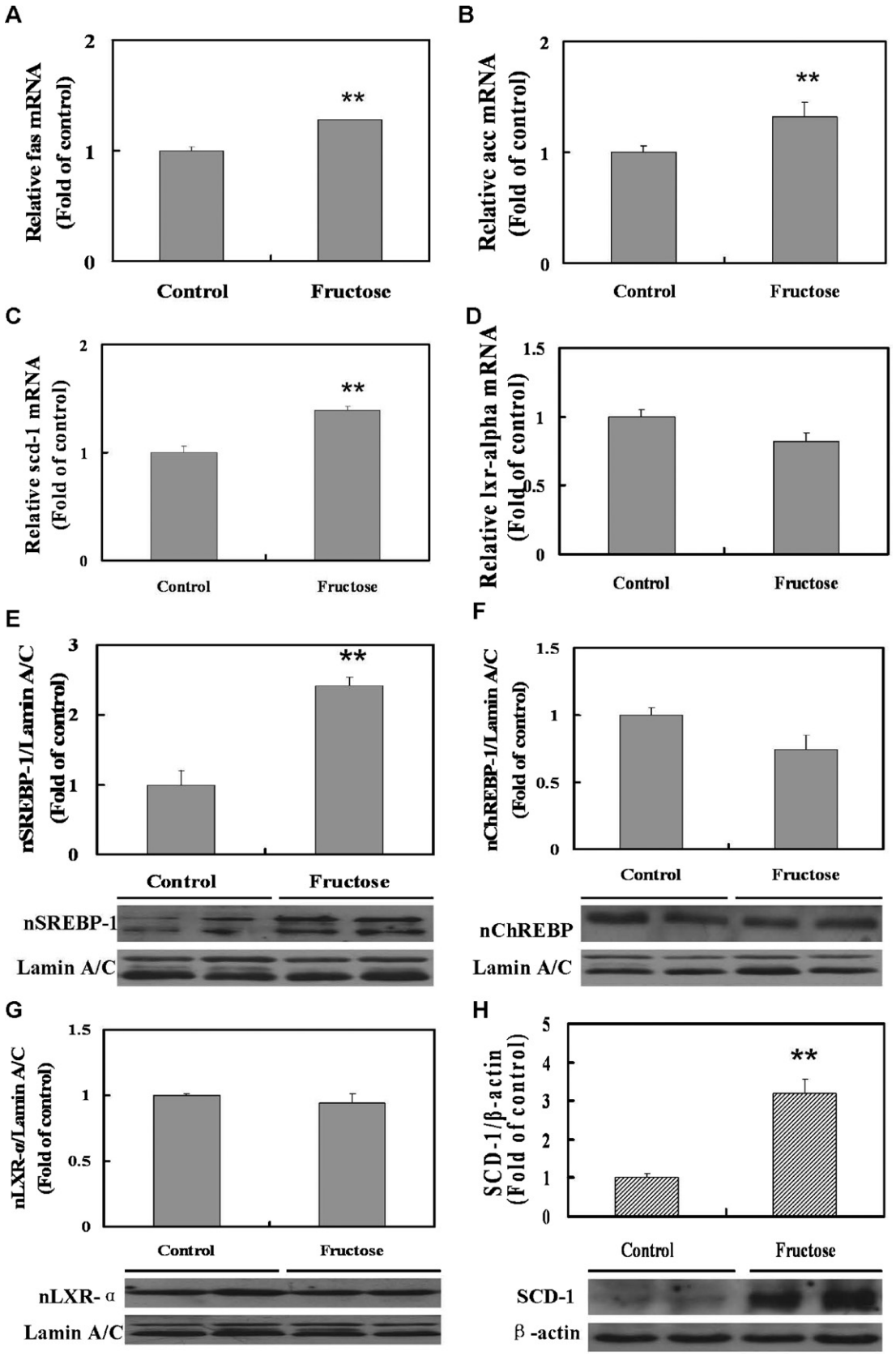

Fig. 2. Chronic fructose drinking activates hepatic SREBP-1c and upregulates the expression of enzymes for fatty acid synthesis. Relative mRNA level of (A) *fas*, (B) *acc*, (C) *scd-1* and (D) *LXR-α* was measured by real-time RT-PCR. Data were expressed as means ± SEM ($n = 6$). (E) Nuclear (n-)SREBP-1, (F) nChREBP and (G) nLXR-α were measured using immunoblots. Blots are representative of three independent experiments. nSREBP-1, nChREBP and nLXR-α were normalized to the level of lamin A/C in the same samples. Data were expressed as means ± SEM ($n = 6$). (H) Hepatic SCD-1 was measured using immunoblots. Blots are representative of three independent experiments. Data were expressed as means ± SEM ($n = 6$). $^{**}P < 0.01$.

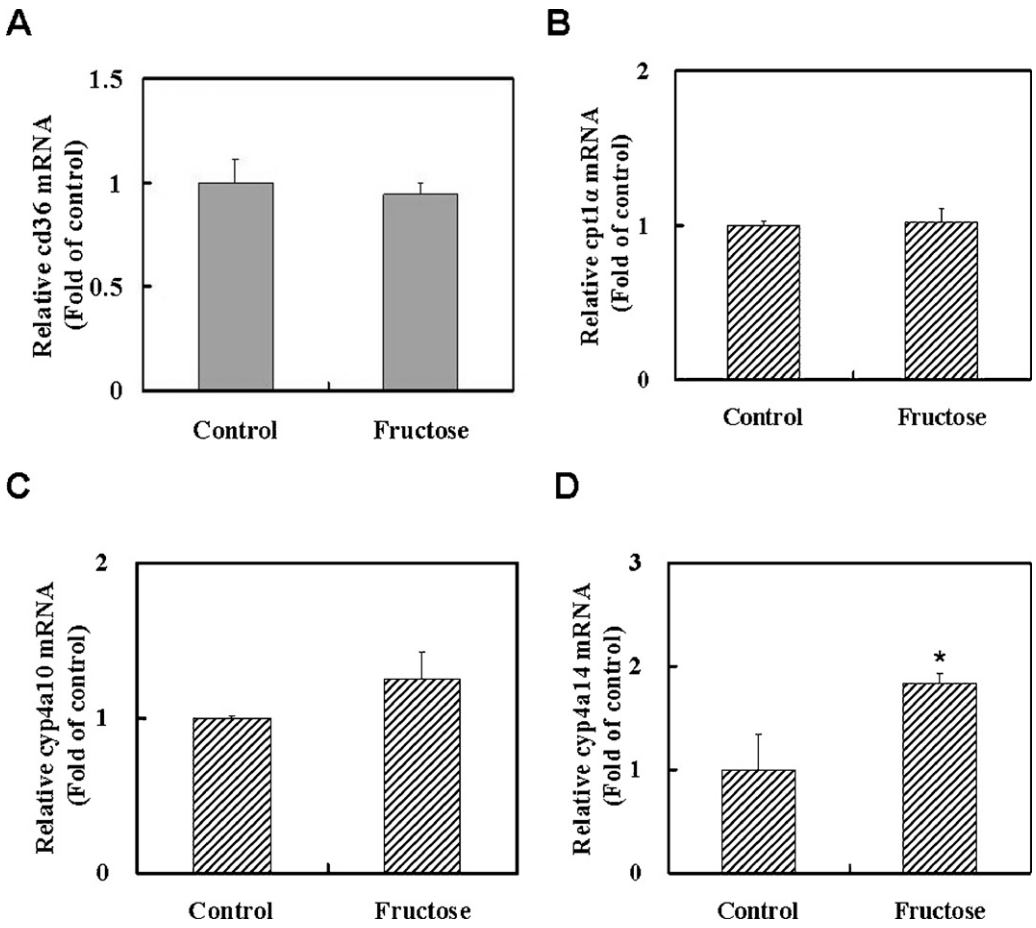

Fig. 3. Effects of chronic fructose drinking on the expression of hepatic *cd36*, *cpt1α*, *cyp4a10* and *cyp4a14*. (A–D) Liver tissues were collected for the preparation of total RNA. Relative mRNA level of (A) *cd36*, (B) *cpt1α*, (C) *cyp4a10* and (D) *cyp4a14* was measured by real-time RT-PCR. Data were expressed as means ± SEM ($n = 6$), $^*P < 0.05$.

3.2. Expression of enzymes for fatty acid synthesis

As shown in Fig. 2A–C, the expressions of hepatic *fas*, *acc* and *scd-1* were significantly upregulated in fructose-fed mice. In addition, the level of hepatic SCD-1 protein was significantly increased in mice fed fructose solution (Fig. 2H). CD36 (also known as fatty acid translocase) is a free fatty acid transporter that is responsible for the uptake of fatty acids from circulation (Schaffer, 2002). As shown in Fig. 3A, chronic fructose drinking had no effect on the expression of hepatic *cd36*. CPT1α is the key regulatory enzyme of hepatic long-chain fatty acid β-oxidation (Gobin et al., 2003). No significant difference on the expression of hepatic *cpt1α* was observed between mice fed with fructose solution and those fed with water (Fig. 3B). CYP4A10 and CYP4A14 are the key enzymes for hepatic ω-oxidation of fatty acids. As shown in Fig. 3C, there was no significant difference on the level of hepatic *cyp4a10* mRNA between fructose-fed mice and controls. The level of hepatic *cyp4a14* mRNA was slightly increased in mice fed with fructose solution (Fig. 3D).

3.3. Hepatic SREBP-1c activation

SREBP-1c and ChREBP are two transcriptional factors that regulate the expression of the enzymes involved in the synthesis of fatty acids (Gobin et al., 2003). As shown in Fig. 2E, the level of hepatic nuclear SREBP-1c was significantly increased in mice fed with fructose solution. However, chronic fructose drinking did not increase the level of hepatic nuclear ChREBP (Fig. 2F). LXRα is an important regulator for hepatic lipid accumulation through directly regulating lipogenic genes, such as *fas*, *acc* and *scd-1* (Lee et al., 2008). As shown in Fig. 2D, chronic fructose drinking did not increase mRNA level of hepatic *lxr-α*. In addition, chronic fructose drinking did not affect the level of hepatic nuclear LXR-α (Fig. 2G).

3.4. Hepatic Insig-1 depletion

Insig-1 and Insig-2 are two important proteins that help SREBP-1 retention in the ER through the interaction with SCAP, thus preventing SREBP-1-SCAP complex translocation to the Golgi apparatus for proteolytic processing (Yang et al., 2002). The effects of chronic fructose drinking on hepatic Insig-1, Insig-2 and SCAP were analyzed. As shown in Fig. 4A and B, chronic fructose drinking significantly upregulated the level of hepatic< insig-1 and insig-2 mRNA. However, the level of hepatic Insig-1 protein was significantly decreased in mice fed with fructose solution (Fig. 4C). Conversely, there was a trend of elevation on hepatic Insig-2 protein in fructose-fed mice (Fig. 4D). Chronic fructose drinking had no effect on the expression of hepatic SCAP (Fig. 4E and F).

3.5. Hepatic ER stress and UPR

The level of hepatic GRP78, an ER chaperone and ATF6 target, was analyzed. As expected, the expression of hepatic GRP78 was significantly increased in fructose-fed mice (Fig. 5D and E). Next, the expression of *trib3*, the target of ATF6, was analyzed. The level of hepatic *trib3* mRNA was significantly increased in mice fed with fructose solution (Fig. 5F). To investigate whether PERK pathway was activated, hepatic PERK phosphorylation was analyzed in mice fed fructose solution. As expected, the level of hepatic pPERK was

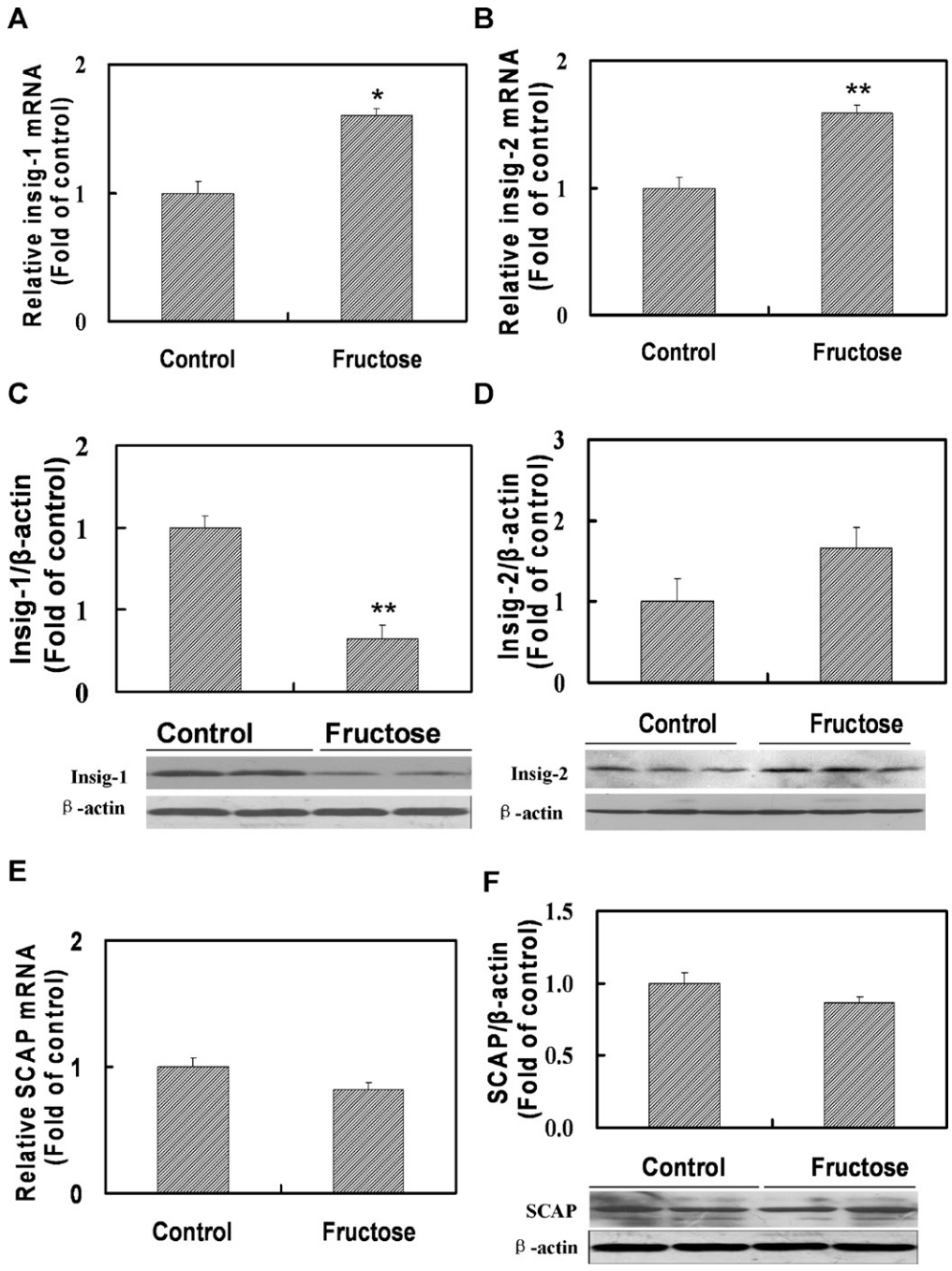

Fig. 4. Effects of chronic fructose drinking on the expression of hepatic Insig1, Insig2 and SCAP. Relative mRNA level of (A) *insig-1*, (B) *insig-2* and (E) *scap*. Data were expressed as means ± SEM ($n = 6$). (C) Hepatic Insig-1, (D) Insig-2, and (F) SCAP proteins were measured by Western blot. Blots are representative of three independent experiments. Data were expressed as means ± SEM ($n = 6$), $^{*}P < 0.05$, $^{**}P < 0.01$.

significantly increased in fructose-fed mice (Fig. 5B). In addition, the level of peIF2α, a downstream target of the PERK pathway, was increased in liver of mice fed with fructose solution (Fig. 5A). To investigate whether hepatic IRE1 pathway was activated in fructose-fed mice, the level of nuclear XBP-1 was measured. As shown in Fig. 5C, the level of hepatic nuclear XBP-1 was significantly increased in mice fed with fructose solution.

3.6. PBA alleviates hepatic ER stress and Insig-1 depletion

The effects of PBA, a chemical chaperone, on fructose-induced hepatic ER stress are presented in Fig. 6. As shown in Fig. 6A and B, PBA significantly attenuated fructose-evoked hepatic PERK and eIF2α phosphorylation. In addition, fructose-induced upregulation of hepatic *trib3* mRNA was significantly alleviated by PBA (Fig. 6C). Moreover, PBA significantly attenuated fructose-evoked hepatic GRP78 upregulation (Fig. 6D). The effects of PBA on hepatic Insig-1 and Insig-2 were analyzed. As shown in Fig. 6E, fructose-induced hepatic Insig-1 depletion was significantly alleviated by PBA, whereas PBA had little effect on hepatic Insig-2 (Fig. 6F).

3.7. PBA inhibits hepatic SREBP-1c activation and lipid accumulation

The effects of PBA on hepatic SREBP-1c activation were analyzed. As shown in Fig. 7A, PBA alone did not affect the level of

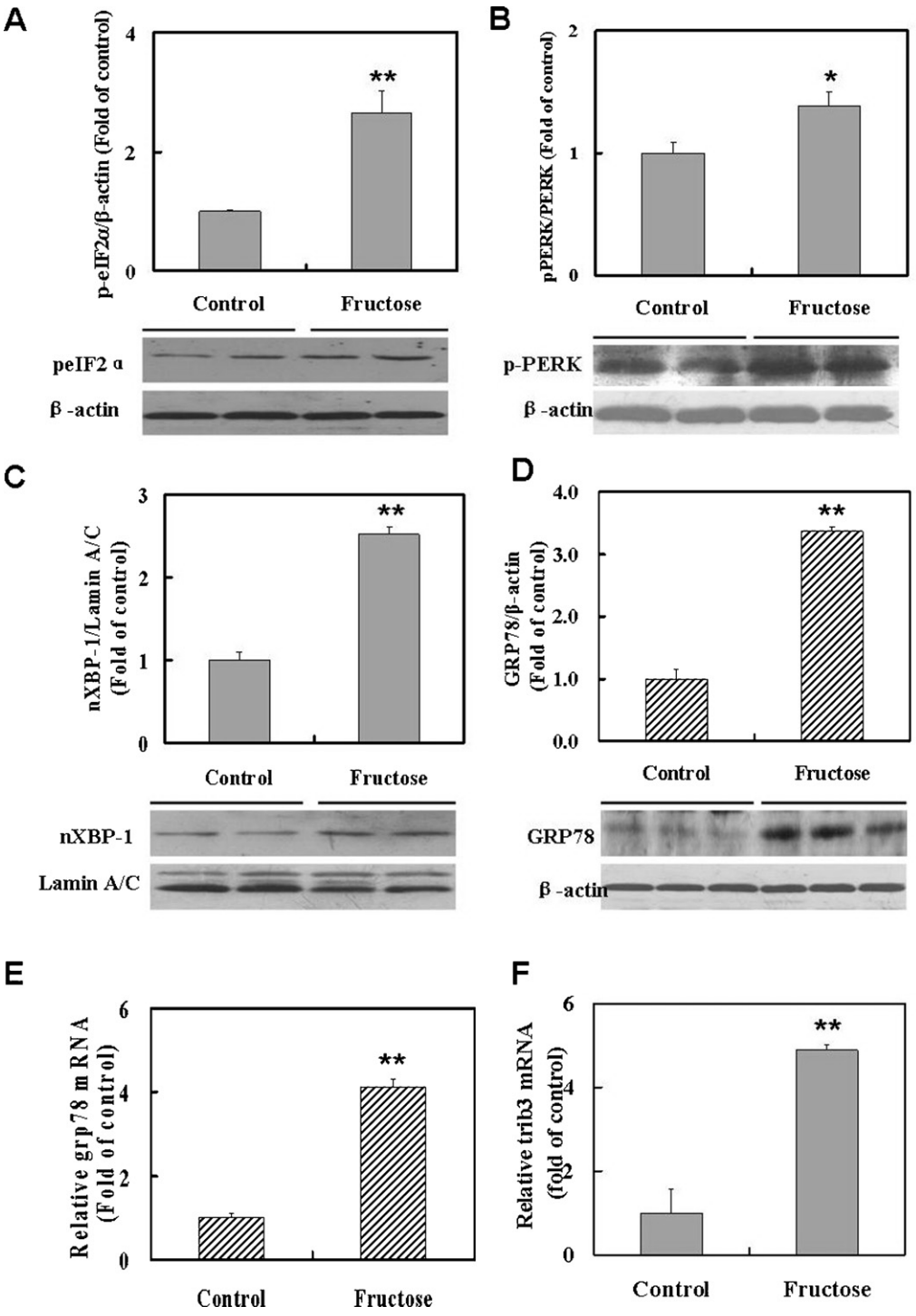

Fig. 5. Chronic fructose drinking induces hepatic ER stress. (A) Hepatic p-eIF2α and (B) pPERK were measured by immunoblots. Blots are representative of three independent experiments. p-eIF2α and p-PERK were normalized to β-actin level in the same samples. Data were expressed as means ± SEM ($n = 6$). (C) Hepatic nXBP-1 and lamin A/C were measured using immunoblots. Blots are representative of three independent experiments. nXBP-1 were normalized to the level of lamin A/C in the same samples. Data were expressed as means ± SEM ($n = 6$). (D) Hepatic GRP78 were measured by immunoblots. Blots are representative of three independent experiments. GRP78 were normalized to β-actin level in the same samples. Data were expressed as means ± SEM ($n = 6$). Relative mRNA level of hepatic (E) *grp78* and (F) *trib3* was measured using real-time RT-PCR. Data were expressed as means ± SEM ($n = 6$), $^{*}P < 0.05$, $^{**}P < 0.01$.

hepatic nuclear SREBP-1c. PBA markedly attenuated the elevation of hepatic nuclear SREBP-1c in mice fed with fructose solution. Correspondingly, fructose-induced upregulation of *srebp-1*, a target of SREBP-1c itself, was alleviated by PBA (Fig. 7F). The effects of PBA on the expression of hepatic fatty acid synthetic enzymes were also analyzed. As shown in Fig. 7C and D, PBA significantly attenuated the upregulation of hepatic *fas* and *acc* mRNA in mice fed with fructose solution. In addition, PBA obviously alleviated fructose-induced upregulation of hepatic *scd-1* (Fig. 7E). The effects of PBA on fructose-induced hepatic lipid accumulation were analyzed. As shown in Fig. 8A, PBA significantly attenuated hepatic lipid accumulation in mice fed with fructose solution. In addition, fructose-induced elevation of serum and hepatic TG was significantly alleviated by PBA (Fig. 8B and C).

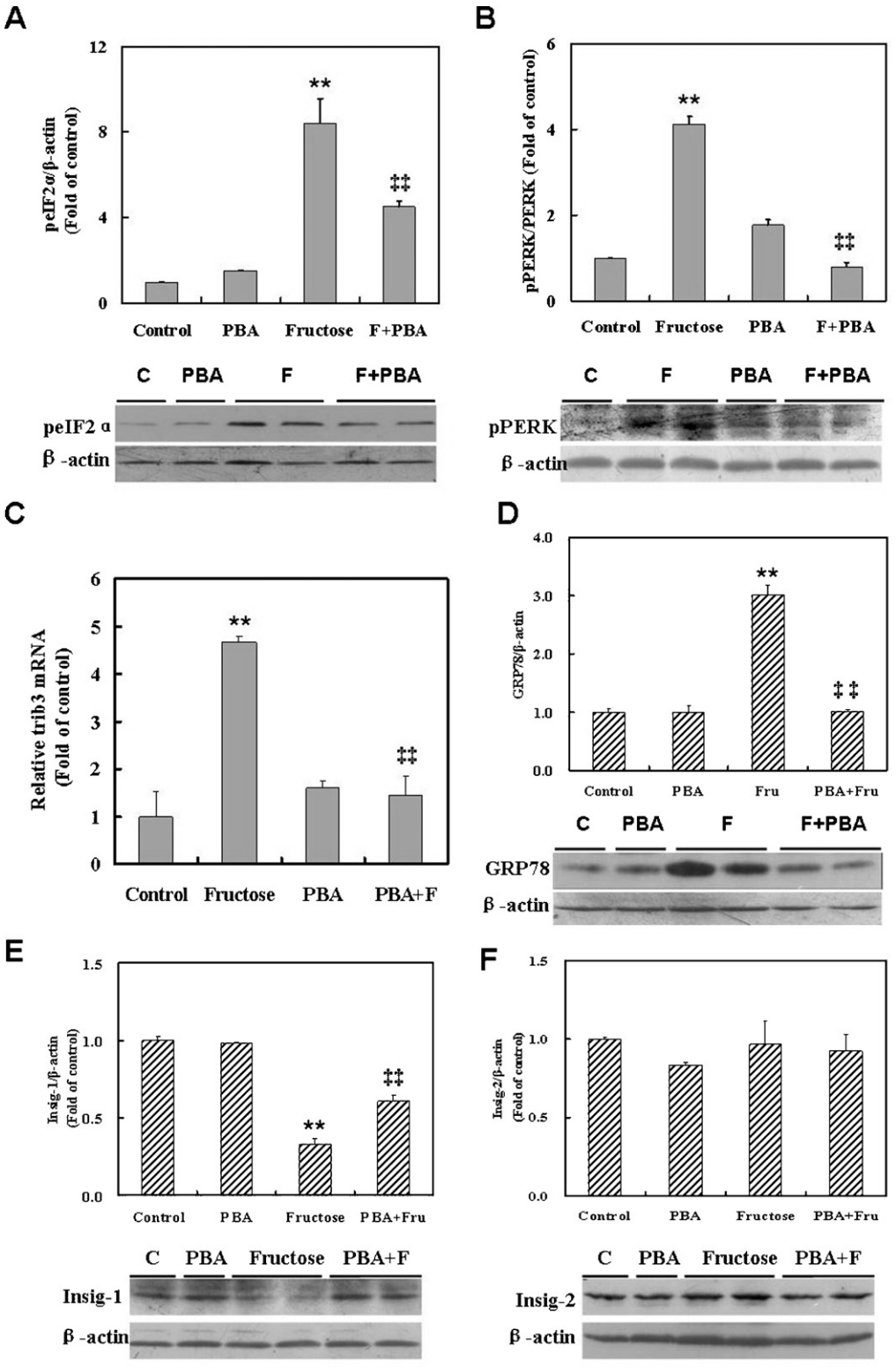

Fig. 6. PBA reduces fructose-induced hepatic ER stress and Insig1 depletion. (A) Hepatic peIF2α and (B) pPERK were measured by immunoblots. Blots are representative of three independent experiments. p-eIF2α and p-PERK were normalized to β-actin level in the same samples. Data were expressed as means ± SEM ($n = 6$). (C) Relative mRNA level of hepatic *trib3* was measured using real-time RT-PCR. Data were expressed as means ± SEM ($n = 6$). (D) Hepatic GRP78 were measured by immunoblots. Blots are representative of three independent experiments. GRP78 were normalized to β-actin level in the same samples. Data were expressed as means ± SEM ($n = 6$). Hepatic (E) Insig-1 and (F) Insig-2 protein was measured by immunoblots. Blots are representative of four independent experiments. Data were expressed as means ± SEM ($n = 6$). $^{**}P < 0.01$ as compared with the control. $^{\ddagger\ddagger}P < 0.01$ as compared with fructose-treated mice.

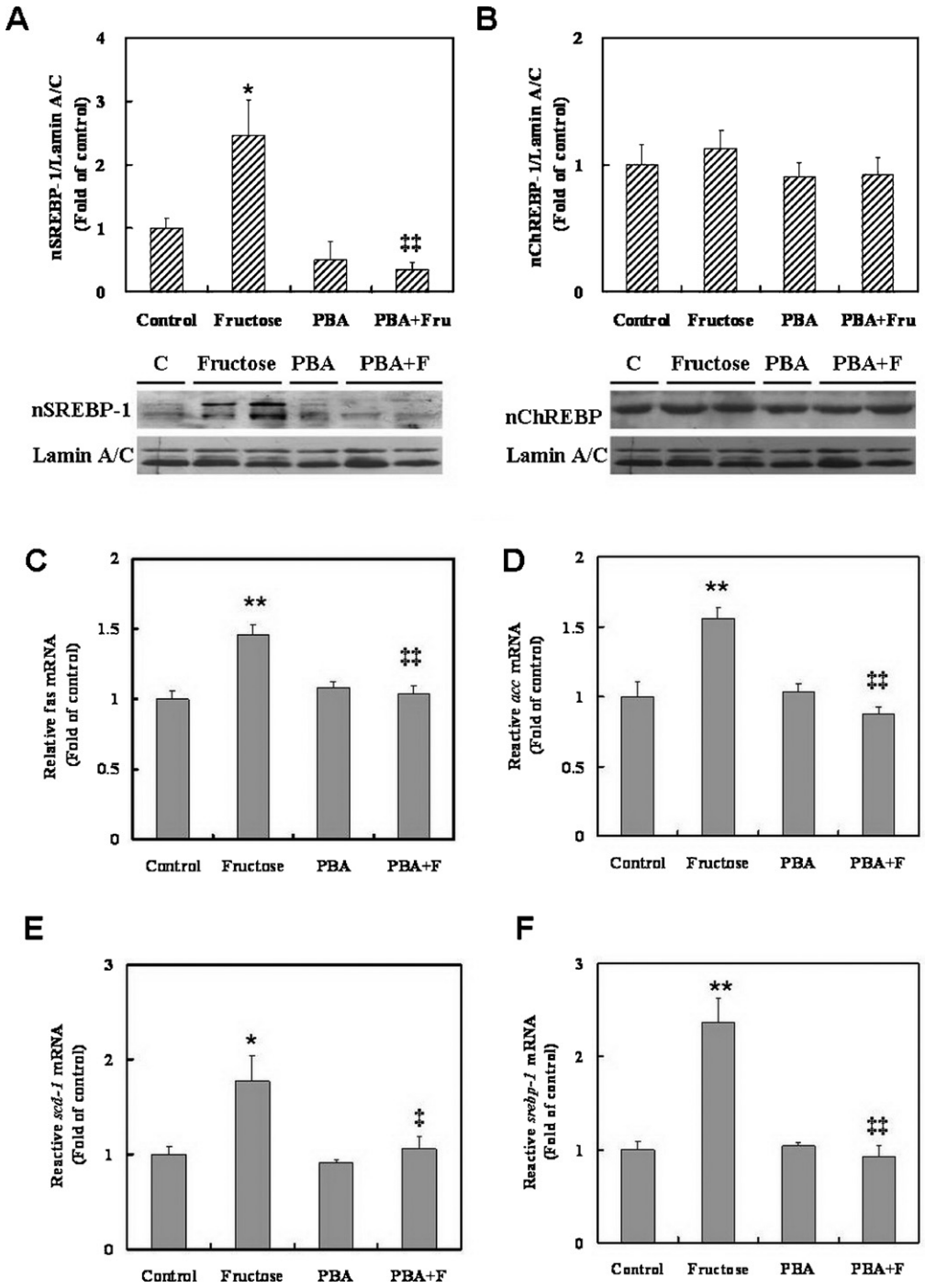

Fig. 7. PBA inhibits hepatic SREBP-1c activation. (A) nSREBP-1 and (B) nChREBP were measured using immunoblots. Blots are representative of three independent experiments. nSREBP-1 and nChREBP were normalized to the level of lamin A/C in the same samples. Relative mRNA level of hepatic (C) *fas*, (D) *acc*, (E) *scd-1* and (F) *srebp-1* was measured using real-time RT-PCR. Data were expressed as means ± SEM ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$ as compared with the control. $^{‡}P < 0.05$, $^{‡‡}P < 0.01$ as compared with fructose-treated mice.

4. Discussion

In the present study, we found that the level of hepatic TG was significantly increased in mice fed with fructose solution. An obvious hepatic lipid accumulation, as determined by Oil Red O staining, was observed in fructose-fed mice. These results are in agreement with several earlier studies, in which the increased level of hepatic TG and the excessive hepatic lipid accumulation was shown in mice fed with fructose solution (Bergheim et al., 2008; Spruss et al., 2009). An increasing evidence demonstrated that de novo fatty acid syntheses might play an important role in the development of NAFLD (Postic and Girard, 2008). In the present study, we found that FAS, ACC and SCD-1, the rate-limiting enzymes for fatty acid synthesis, were significantly upregulated in mice fed with fructose solution. By contrast, chronic fructose drinking had no effect on the expression of CD36, a free fatty acid transporter that is responsible for the uptake of fatty acids from circulation, and CPT1α, CYP4A10 and CYP4A14, the key enzymes for fatty acid oxidation. Thus, the elevation of de novo fatty acid syntheses rather than the uptake of fatty acids from circulation or the decreased oxidation of fatty

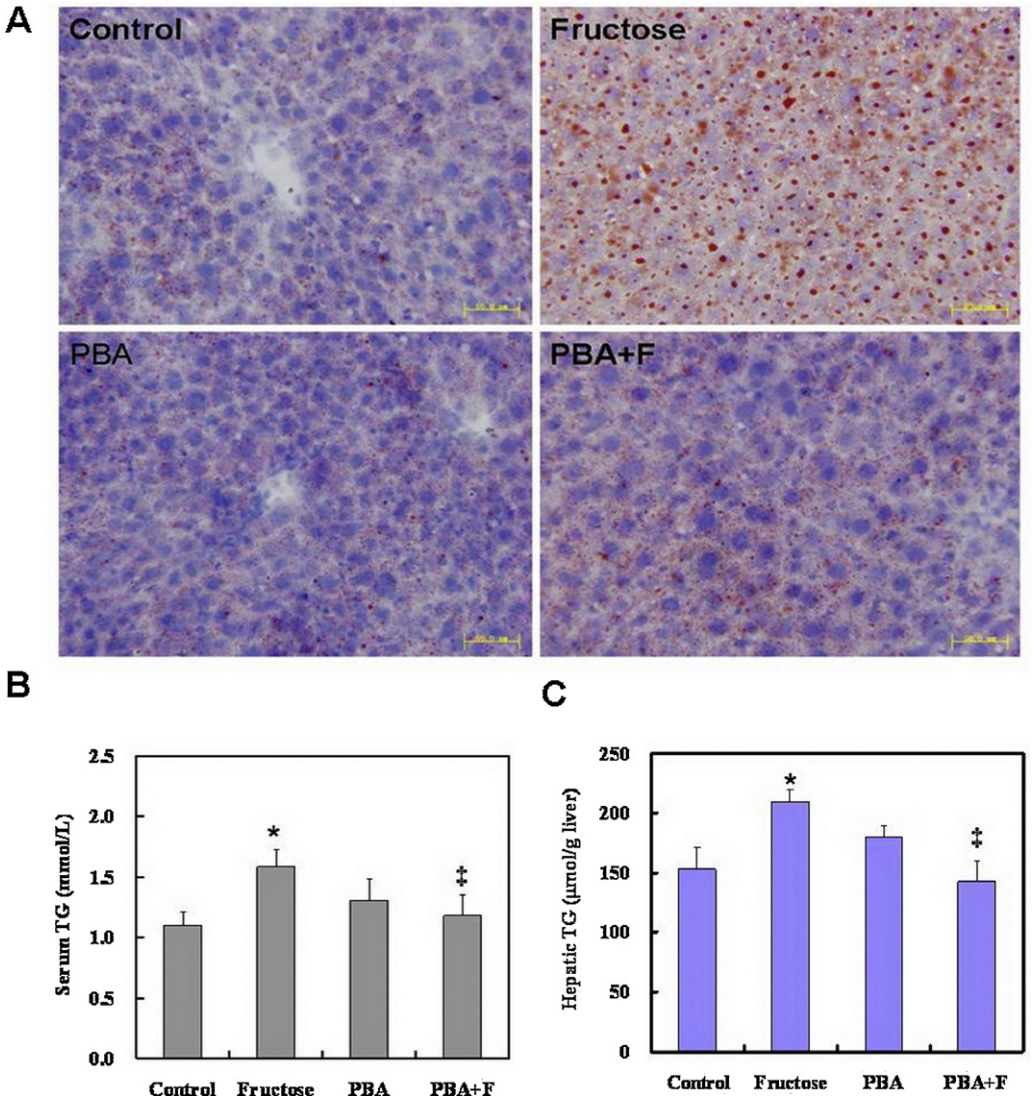

Fig. 8. PBA protects against hepatic lipid accumulation. (A) Liver sections were stained with Oil Red O. Original magnification, 400×. (B) Serum and (C) hepatic triglycerides (TG) contents. Data were expressed as means ± SEM of twelve samples. $^{*}P < 0.05$ as compared with the control. $^{‡}P < 0.05$ as compared with fructose-treated mice.

acids contributes to hepatic lipid accumulation in fructose-induced NAFLD.

SREBP-1c is the most important transcription factor that regulates the expression of the enzymes for fatty acid synthesis. Several studies found that the mature form of SREBP-1c was significantly increased in liver of obese *ob*/*ob* mice, a model of NAFLD in which the expression of hepatic *fas* and *scd-1* was up-regulated (Kammoun et al., 2009; Sekiya et al., 2003; Shimomura et al., 1999). The present study found that the level of hepatic nuclear SREBP-1c was significantly increased in mice fed with fructose solution. In addition, the expression of hepatic *srebp-1*, the target of active SREBP-1c itself, was significantly upregulated in fructose-fed mice. In addition to SREBP-1c, ChREBP is another important transcription factor that regulates the expression of the enzymes for fatty acid synthesis (Postic et al., 2007; Uyeda and Repa, 2006). A recent study showed that liver-specific inhibition of ChREBP markedly improved hepatic steatosis by specifically decreasing lipogenic rates in ob/ob mice (Dentin et al., 2006). However, no significant difference on the level of nuclear ChREBP was observed between fructose-fed mice and controls. LXR-α is also an important transcription factor that regulates the expression of the enzymes for fatty acid synthesis through direct interaction with the FAS promoter as well as through activation of SREBP-1c expression (Joseph et al., 2002; Yoshikawa et al., 2001). In the present study, we showed that no significant difference on mRNA level of hepatic LXR-α was observed between fructose-fed mice and controls. In addition, chronic fructose drinking had no effect on the level of nuclear LXR-α in liver. Taken together, these results suggest that activation of SREBP-1c but not ChREBP or LXR-α might contribute to the increased expression of enzymes for fatty acid synthesis in liver of mice fed with fructose solution.

An increasing evidence demonstrated that ER stress plays an important role for hepatic SREBP-1c activation and the development of NAFLD. According to an earlier report, ER stress was associated with hepatic SREBP-1c activation in homocysteine-induced NAFLD (Werstuck et al., 2001). A recent study found that ER stress mediated hepatic SREBP-1c cleavage in obese *ob*/*ob* mice (Kammoun et al., 2009). The present study found that for the first time that ER stress and the UPR signaling were activated in liver of mice fed with fructose solution. First, GRP78, an important molecular chaperone, was significantly upregulated in liver of mice fed with fructose solution. Second, an increased hepatic PERK and

eIF2α phosphorylation was observed in mice fed fructose solution. Third, the level of active XBP-1 was significantly increased in hepatic nuclear extracts of mice fed with fructose solution. Finally, TRIB3, the target of ATF6 pathway, were significantly upregulated in liver of mice drinking fructose solution. PBA is a chemical chaperone that stabilizes protein conformation and improves ER protein folding capacity. An earlier study showed that PBA alleviated ER stress and restored glucose homeostasis in a mouse model of insulin resistance (Ozcan et al., 2006). A recent study found that PBA alleviated ER stress and protected mice from the development of leptin resistance in obese *ob/ob* mice (Ozcan et al., 2009). To investigate the role of ER stress in fructose-induced SREBP-1c activation, the effects of PBA on fructose-induced SREBP-1c activation were analyzed. As expected, PBA significantly alleviated fructose-evoked hepatic PERK and eIF2α phosphorylation. In addition, PBA significantly attenuated fructose-induced upregulation of TRIB3 and GRP78 in liver. Importantly, PBA administration almost completely inhibited hepatic SREBP-1c activation. Correspondingly, PBA significantly attenuated the upregulation of SREBP-1c target genes, reduced TG accumulation in liver of mice fed with fructose solution. These results suggest that ER stress and the UPR pathway might be involved in fructose-induced SREBP-1c activation and hepatic TG accumulation.

How ER stress and the UPR mediate hepatic SREBP-1c activation remains obscure. Insig-1 is an ER protein that binds the sterol-sensing domain of SCAP and facilitates retention of the SCAP/SREBP complex in the ER (Yang et al., 2002). Several reports demonstrated that overexpression of Insig-1 repressed hepatic SREBP-1c activation and lipogenesis in different models of NAFLD (Engelking et al., 2004; Takaishi et al., 2004). A recent study found that PPARδ agonist or overexpression of PPARδ inhibited the proteolytic processing of SREBP-1 into the mature active form and reduced hepatic lipogenesis in obese diabetic mice through upregulation of hepatic Insig-1 (Qin et al., 2008). On the other hand, protein synthesis is inhibited during the UPR owing to phosphorylation of eIF2α, a translation initiation factor. As shown in the present study, the level of hepatic Insig-1 protein was significantly decreased in mice fed fructose solution. Thus, we hypothesize that reduction of hepatic Insig-1 protein might play an important role in fructose-evoked hepatic SREBP-1c activation. Indeed, an earlier study demonstrated that SREBP-1c was activated by ER stress and the UPR through depletion of Insig-1 protein (Lee and Ye, 2004). To further demonstrate whether depletion of Insig-1 protein mediates fructose-evoked hepatic SREBP-1c activation, the effects of PBA on fructose-induced hepatic Insig-1 depletion were investigated. Surprisingly, fructose-induced hepatic Insig-1 depletion was significantly alleviated by PBA. These results suggest that hepatic Insig-1 depletion, induced by ER stress and the UPR, contributes, at least partially, to fructose-evoked hepatic SREBP-1c activation.

In summary, the present study demonstrated that hepatic SREBP-1c activation and subsequent de novo fatty acid syntheses rather than the uptake of fatty acids from circulation or the decreased oxidation of fatty acid contributes, at least in part, to hepatic lipid accumulation in fructose-induced NAFLD. Chronic fructose drinking activates hepatic ER stress and UPR signaling, which might play an important role on fructose-evoked hepatic Insig1 depletion and SREBP-1c activation. Importantly, PBA, an ER chemical chaperone, can effectively protect mice from hepatic SREBP-1c activation and lipid accumulation in fructose-induced NAFLD.

Conflict of interest

The authors declare that there are no conflicts of interest.

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