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Dietary inulin alleviates hepatic steatosis and xenobiotics-induced liver injury in rats fed a high-fat and high-sucrose diet: Association with the suppression of hepatic cytochrome P450 and HNF4α expression

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E) ABBREVIATIONS: C, vehicle; CAR, constitutive androstane receptor; DEX, dexamethasone; HF, high fat and high sucrose diet; HF + I, inulin-supplemented HF; HNF, hepatocyte nuclear factor; CYP, cytochrome P450; PB, phenobarbital; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1α; PROD, pentoxyresorufin *O*-dealxylase; PXR, pregnane X receptor; PCR, polymerase chain reaction; RXRα, retinoid X receptor α; SD, standard diet, SD + I, inulin-supplemented SD; TBARS, thiobarbituric acid-reactive substances; TNF, tumor necrosis factor.

Abstract

Inulin enzymatically synthesized from sucrose is a dietary component that completely escapes glucide digestion. Supplementing inulin to a high-fat and high-sucrose diet (HF) ameliorated hypertriglycemia and hepatic steatosis in 8-week-fed rats by suppressing elevated levels of serum triacylglycerols, fatty acids and glucose, and the accumulation of hepatic triacylglycerols and fatty acids. Inulin intake prevented phenobarbital (PB)- and dexamethasone-induced liver injuries in the HF group. No significant alteration in the baseline expression of CYP2B, CYP2C11, CYP3A and NADPH P450 reductase mRNAs and proteins was found. In contrast, baseline and PB-treated expressions of CYP2E1 mRNA were reduced in HF-fed rats. The induction of CYPs in response to PB was affected by their nutritional status; mRNA levels of CYP2B1 and CYP3A1 after PB treatment as assessed by quantitative real-time PCR analysis were reduced in the inulin-supplemented HF (HF+I) group, compared with those in the HF group. Western-blot analysis detected the corresponding changes of CYP2B and CYP3A proteins. These alterations were correlated with changes in hepatic thiobarbituric acid-reactive substances. Furthermore, no significant difference in the expression of nuclear receptors CAR, PXR, and RXRα and coactivator PGC1α proteins was found in the hepatic nucleus between HF and HF+I groups, but the expression of HNF4α protein was significantly reduced in the HF+I group. Taken together, these results indicate that inulin intake ameliorates PB-induced liver injury, associated with a decline in lipid accumulation and PB-induced expression of CYP2B and CYP3A, which may be related by a reduction in the nuclear expression of HNF4α.

Introduction

The liver plays a central role in metabolizing therapeutic drugs and environmental contaminants. The activities of drug-metabolizing enzymes in our body are affected by the genotypes of the translating gene and also by non-genetic factors including environmental factors. Nutritional status such as starvation, fasting and high-lipid diet, and pathophysiological alterations such as diabetes have been reported to modulate liver microsomal cytochrome P450 (CYP) composition, leading to altered hepatic metabolism of drugs, carcinogens, steroid hormones, and fatty acids. Uncontrolled diabetes enhances hepatic CYP2E1, CYP2B, CYP3A and CYP4A expression (Woodcroft et al., 1999). An increase in the hepatic production and accumulation of triacylglycerols is frequently associated with metabolic syndromes such as hypertriglyceridemia, obesity and insulin resistance. The accumulation of triacylglycerols, defined as hepatic steatosis, is proposed to be the setting for more severe liver diseases such as nonalcoholic steatohepatitis possessing histologic signs of fibrosis and necroinflammation through to cirrhosis, terminal liver failure and hepatocellular carcinoma (Bugianesi et al., 2002). CYP2E1, a microsomal oxidase involved with fatty acid ω-oxidation as well as CYP4A, is upregulated in nutritional statuses such as starvation, fasting, obesity and hyperlipidemia (Hong et al., 1987; Johansson et al., 1988; Weltman et al., 1996; Leclercq et al., 2000). The elevated expression of CYP2E1 has been largely attributed to the pathogenesis of liver disease in patients with nonalcoholic steatohepatitis (Weltman et al., 1996, 1998; Leclercq et al., 2000; Emery et al., 2003; Chalasani et al., 2003).

Biochemical processes that generate oxidative stress can initiate hepatocellular injury, and CYP2B, CYP2C and CYP3A as well as CYP2E1 and CYP4A are

microsomal sources of producing prooxidants in liver cells, which create oxidative stress under uncoupling with antioxidants. Here we found that the intake of a high-fat and high-sucrose diet (HF) for a long period of 8 weeks produced marked accumulation of hepatic and serum triacylglycerols in rats, leading to hypertriglycemia and hepatic steatosis but not hepatic necroinflammatory lesions. Also, there was no significant influence in the baseline levels of hepatic CYP2B, CYP2C and CYP3A proteins, but levels of CYP2E1 and CYP4A proteins were reduced. In contrast, the administration of phenobarbital (PB) to HF-fed rats more promptly caused liver injury leading to cell necrosis and inflammation, compared to the standard diet (SD)-fed rats, and was associated with changes in PB-inducible CYP expression or via the CAR-mediated pathway associated with hepatic lipid accumulation.

Some dietary components that completely escape glucide digestion, such as resistant starch and oligofructose, have been demonstrated to exert systemic effects by modifying lipid metabolism (Younes et al., 1995; Daubiou et al., 2000). In contrast to starch, inulin is fermentable dietary fiber, resistant to hydrolysis by pancreatic amylase and saccharidases in the upper gastrointestinal tract. Previous studies by our laboratory have demonstrated that we produced inulin enzymatically from sucrose, and that supplementing inulin to the HF diet for 12 weeks reduced elevated body weight and serum and hepatic levels of triacylglycerols in rats (Wada et al., 2005). Interestingly, this study has demonstrated that PB-induced liver failure was suppressed in rats fed an inulin-supplemented HF diet. It is noteworthy to investigate whether the reduction in elevated levels of blood glucose, serum and hepatic lipids by supplementing inulin to the HF diet was associated with the down-regulation of CYP induction by PB, consequently leading to the suppression of liver injury. Hence, in this study, we

characterized PB-induced CYP mRNA and protein profiles in connection with profiles of blood glucose, serum and hepatic lipids. Treatment of inulin-supplemented HF diet-fed rats with PB resulted in lower levels of CYP2B mRNA and protein than with only the SD or HF diet. PB induces CYP2B, CYP2C and CYP3A via the activation of nuclear receptors, constitutive androstane receptor (CAR) and pregnane X receptor (PXR), which regulate the transactivation of those genes. Previous studies have demonstrated that hepatocyte nuclear receptor-4α (HNF4α) is an important regulator of coordinate nuclear receptor (such as CAR and PXR)-mediated response to xenobiotics (Triona et al., 2003), and that peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α) modulates the function of nuclear receptors such as CAR as a common coactivator (Shiraki et al., 2003: Puigserver and Spiegelman, 2003). Little is known about the effects of nutritional status on baseline and xenobiotic-mediated expression of nuclear receptors (CAR, PXR, RXRα forming a heterodimer with CAR or PXR) and coactivator PGC1α. Thus, we examined the effect of supplementing inulin to SD and HF diets on the hepatic expression of CAR, PXR, RXRα, HNF4α and PGC1α mRNAs and nuclear expression of those proteins in rats.

Materials and Methods

Materials. Phenobarbital sodium (PB) was purchased from Wako Pure Chemicals (Osaka, Japan). Dexamethasone (DEX), pentoxyresorufin, and resorufin were from Sigma Aldrich (St. Louis, MO). Inulin, which consists of a linear polymer (average ratio of glucose/fructose, 1:17) having β(2-1) linkages of D-fructose with one terminal glucose, was synthesized enzymatically from sucrose by inulin-producing enzyme as described previously (Wada et al., 2005). All other chemicals and solvents were of analytical grade and were obtained from commercial sources.

Experimental animals. All studies followed protocols approved by the Institutional Animal Care and Life Committee, University of Shizuoka. Male Wistar rats were obtained from Japan SLC (Hamamatsu, Japan) at 4 weeks of age and were acclimatized for one week prior to the experiment. Animals were housed in stainless-steel hanging cages with free access to food and water and maintained on a 12-h light-dark cycle. All animals were randomly assigned to standard diet (SD), 5% inulin-supplemented standard diet (SD+I), high-fat and high-sucrose diet (HF), or 5% inulin-supplemented high-fat and high-sucrose diet (HF+I) for 4 and 8 weeks. The HF diet consisted of 19.7% casein, 1% soybean oil, 10% lard, 4% mineral mixture, 1% vitamin mixture, 0.15% choline chloride, 0.5% cholesterol, 0.25% sodium cholate, 3.4% cellulose and 60% sucrose (23.9% lipid, 56.8% carbohydrate and 19.3% protein [kJ]), and the SD diet consisted of 23.8% crude protein, 5.1% crude fat, 3.2% crude fiber, 6.1% ash, 54% nitrogen-free extract and 7.8% humidity (12.9% lipid, 60.4% carbohydrate and 26.7% protein [kJ]). Three times per week, all rats were weighed, and food intake in grams was monitored. At 9 or 13 weeks of age the rats in each group were administered PB (80 mg/kg) or vehicle (0.9% sodium chloride) by intraperitoneal injection once daily for

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4 days and dexamethasone (50 mg/kg) or vehicle (0.1 M citric acid in 0.5% methylcellulose) once daily for 3 days between 8:00 AM and 9:00 AM.

Blood and tissue sampling. The experiments were run 4 h after the last administration of PB. Rats were anesthetized with diethyl ether, then the abdominal cavity was rapidly opened, and blood was rapidly and simultaneously drawn from the abdominal vena cava into syringes. Plasma samples were separated from blood collected into heparinized tubes by centrifugation, and serum samples were separated from blood by centrifugation after standing for 30 min at room temperature. The resulting plasma/serum was stored at –30^{o}C until analysis. Livers were rapidly excised and weighed. The liver median lobe was excised for the preparation of microsomes and nuclear extracts and RNA extraction, or was fixed in 4% paraformaldehyde in 0.12 M sodium phosphate (pH 7.3) for histological examination. Aliquots were snap frozen and stored at –80^{o}C until analysis. Sections were stained with hematoxylin-eosin (HE) or Oil Red O.

Preparation of microsomes. Liver microsomes were prepared by differential centrifugation, first at 9,000g for 15 min followed by 105,000g for 60 min at 4^{o}C and stored at –80^{o}C. The microsomal protein concentration was determined with a bicinchoninic acid protein assay kit (Pierce Chemical, Rockford, IL) using bovine serum albumin as a standard.

Preparation of nuclear extracts. Livers (about 1 g) were homogenized in 5 volumes of 10 mM HEPES-NaOH buffer (pH 7.6) containing 25 mM KCl, 2 M sucrose, 10% glycerol, 0.15 M spermine, 0.5 mM spermidine, 1 mM EDTA, 1 mM dithiothreitol, 1 μg of leupeptin per ml, 1 μg of pepstatin per ml, 1 μg of aprotinin per ml, 0.1 mM phenylmethylsulfonyl fluoride, and 1 mM Na_3VO_4 with a Teflon-glass homogenizer

with 3 strokes. The homogenate was carefully layered onto 5 ml of the same buffer in a centrifuge tube, and this was followed by centrifugation at 25,000 rpm and 4°C for 60 min with an SW28 rotor (Himac CP56GII, Hitachi Koki, Hitachinaka, Japan). The precipitate was homogenized in 1 ml of lysis buffer (10 mM HEPES-NaOH buffer [pH 7.6] containing 0.1 M KCl, 3 mM $MgCl_2$, 10% glycerol, 0.1 mM EDTA, 1 mM dithiothreitol, 1 μg of leupeptin per ml, 1 μg of pepstatin per ml, 1 μg of aprotinin per ml, 0.1 mM phenylmethylsulfonyl fluoride, and 1 mM Na_3VO_4) with a Dounce homogenizer 10 times. Sodium chloride solution was added to the homogenate to bring the final concentration to 0.4 M, and the homogenate was incubated at 4°C for 30 min with gentle shaking. The supernatant of the centrifugation at 105,000g for 20 min was dialyzed against 1 L of dialysis buffer (20 mM HEPES-NaOH buffer [pH 7.6] containing 0.1 M NaCl, 20% glycerol, 0.2 mM EDTA, 1 mM DTT, 1 μg of leupeptin per ml, 1 μg of pepstatin per ml, 1 μg of aprotinin per ml, 0.1 mM phenylmethylsulfonyl fluoride, and 1 mM Na_3VO_4) at 4°C overnight and stored at –80°C. The protein concentration was determined by the Bradford assay (Bio-Rad protein assay kit, Bio-Rad Laboratories, Hercules, CA) using bovine serum albumin as a standard.

Immunoblot analysis. Microsomal proteins or nuclear extracts (20 μg) were resolved on a sodium dodecyl sulfate-12.5% polyacrylamide gel, electroblotted onto a polyvinylidene difluoride membrane (Millipore Corporation, Bedford MA). The immunoblots were incubated with the following primary antibodies: rat CYP2B1, rat CYP2C11, rat CYP2E1, rat CYP3A2, rat CYP4A1, and rat NADPH P450 reductase (Daiichi Pure Chemicals Co., Tokyo, Japan), mouse CAR, mouse PXR, human RXRα, human HNF4α, human PGC1α, and human histone H1 (Santa Cruz Biotechnology,

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Santa Cruz, CA). Antigen-antibody complexes were detected using the appropriate secondary antibody conjugated with horseradish peroxidase and visualized with an enhanced chemiluminescence system (GE Healthcare Bio-Sciences, Piscataway, NJ).

Determination of mRNA levels. Total RNA was prepared from the liver using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA). Samples were quantitated by spectrophotometry, and 1 μg of total RNA was used to generate cDNA by reverse transcription (RT) using SuperScript II (Invitrogen) according to the manufacturer's protocol. cDNA synthesized from 50 ng of total RNA was subjected to quantitative real-time polymerase chain reaction (PCR) as described previously (Sugatani et al., 2005) with an ABI PRISM 7000 Sequence Detector instrument (Applied Biosystems, Foster City, CA) using Premix Ex Taq reagent (TaKaRa Bio Inc., Otsu, Japan) for TaqMan probe method or SYBR Premix Ex Taq reagent (TaKaRa Bio Inc.) for intercalation reaction with SYBR Green I according to the manufacturer's specifications. The TaqMan probes and primers for rat *CAR* and *PXR* were as reported previously (Hartley et al., 2004), and rat *CYP2E1* (assay identification number Rn00580624_m1), rat *HNF4α* (assay identification number Rn00573309_m1) and rat *β-actin* (assay identification number Rn00667869_m1) were assay-on-demand gene expression products (Applied Biosystems). The gene-specific probes were labeled by using reporter dye FAM, and the β-actin internal control probe was labeled with a different reporter dye VIC at the 5' end. The primers for rat CYP2B1, rat CYP2B2, rat CYP3A1, and rat CYP3A2 (Yoshinari et al., 2004), rat CYP2C11 (Xu et al., 2001), rat CYP4A1, rat CYP4A2, rat CYP4A3 (Marji et al., 2002), rat RXRα (Wang et al., 2002), and rat PGC1α (Zhang et al., 2005) were as reported previously, the primers for rat NADPH cytochrome P450 reductase were 5'-CATGTCCGCAGACCCTG-AA-3'

(forward primer) and 5'-CGTATGTGGCCATGC-AGAAG-3' (reverse primer), and the primers for rat β-actin were 5'-TCCTAGCACCATGAAGATC-3' (forward primer) and 5'-AAACGCAGCTCAGTAACAG-3' (reverse primer). The thermal cycle conditions were as follows: hold for 10 sec at 95°C, followed by two-step PCR for 40 cycles of 95°C for 5 sec followed by 60°C for 30 sec. β-Actin was used to normalize gene expression in all samples. Fold-induction values were calculated by subtracting the mean difference of gene and β-actin cycle threshold Ct numbers for each treatment group from the mean difference of gene and β-actin Ct numbers for the vehicle group and raising the difference to the power of 2 ($2^{-\Delta\Delta Ct}$).

Biochemical analyses. Plasma concentrations of glucose and fructose were determined by the hexokinase method using commercial reagents (R-Biopharm AG, Darmstadt, Germany). Serum glucose concentration was determined by the glucose oxidase method with a commercially available kit from Shino Test (Tokyo, Japan). Plasma/serum insulin was measured using a double antibody RIA kit specific rat insulin (Eiken Chemical Co., Tokyo, Japan). Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyltranspeptidase (γ-GTP) were measured using kits from Wako Pure Chemicals (Osaka, Japan). Serum levels of triacylglycerol, total cholesterol, and nonesterified fatty acid were measured enzymatically with kits from Shino Test. Serum concentrations of β-hydroxybutyrate were measured using commercial reagents (Serotec Co., Sapporo, Japan). Frozen livers (about 0.5 g) were homogenized in 20 volumes (the SD and SD+I groups) and 100 volumes (the HF and HF+I groups) of 0.9% NaCl containing 0.1% Triton X-100, and the concentrations of triacylglycerol, total cholesterol, and nonesterified fatty acid were estimated with the

above commercial kits. Frozen livers (about 0.5 g) were homogenized in 100 volumes of 0.9% NaCl and thiobarbituric acid-reactive substances (TBARS) were measured in 20 μl liver homogenate using a commercial assay kit (ZeptoMetrix Co., Buffalo, NY). Liver tumor necrosis factor (TNF) α and interleukin 6 were measured in 12.5 μl serum or liver homogenate using commercial ELISA kits (GE Healthcare Bio-Sciences).

Resorufin assay. The *O*-dealkylation of 7-pentoxyresorufin was measured fluorometrically by the production of resorufin using a Wallac 1420 ARVO plate reader (PerkinElmer Inc., Wellesley, MA) with excitation and emission wavelengths at 550 nm and 590 nm, respectively. The incubation mixtures contained Tris-HCl (100 mM, pH 7.4), $MgCl_2$ (3.3 mM), EDTA (1 mM), glucose-6-phosphate (3.3 mM), NADP (1.3 mM), glucose-6-phosphate dehydrogenase (0.4 U/ml), pentoxyresorufin (5 μM) and microsomal protein from control rats and PB-treated rats (25 and 2.5 μg/ml, respectively). NADPH was produced using the GENTEST NADPH regenerating system (BD Biosciences, Woburn, MA). The mixtures were incubated for 10 min at 37^{o}C. The formation rate of resorufin was determined by comparison to the fluorescence of known amounts of resorufin.

Statistics. Values are expressed as the mean $\pm$ standard error. All data were analyzed using one-way analysis of variance. The difference between the means of the groups was tested for significance using Student's t-test. The level of statistical significance was set at $p<0.05$.

Results

Dietary inulin ameliorates the development of fatty livers in rats fed high-fat- and high-sucrose-supplemented diet for 8 weeks.

We previously reported that supplementing inulin for 12 weeks reduced the elevation in body weight and serum and hepatic levels of triacylglycerols in rats fed high-fat (10% lard) and high-sucrose (60%) diet (HF), whereas inulin intake for 12 weeks in rats fed the SD diet containing naturally existing starch as carbohydrate did not affect the body weight and serum and hepatic levels of lipids (Wada et al., 2005). Consistent with these observations, supplementing inulin even for 8 weeks reduced the elevation of body weight, liver weight, serum levels of triacylglycerols, fatty acids and glucose, and hepatic levels of triacylglycerols in rats fed the HF diet, but did not significantly alter body weight, liver weight, and serum levels of lipids and glucose in rats fed the SD diet (Table 1). At this time, there were no significant differences between the 4 diet groups in the food intake and starting body weight (data not shown). No significant change in serum and hepatic levels of total cholesterol was found by inulin intake (Table 1). In contrast, the hepatic levels of triacylglycerol (72.6 $\pm$ 8.7 mg/g liver) in rats fed the HF diet for 8 weeks were significantly elevated compared to those of rats fed the HF diet for 4 weeks (43.8 $\pm$ 5.0 mg/g liver), and supplementing inulin to the HF diet suppressed the accumulation of hepatic triacylglycerols (44.8 $\pm$ 5.8 mg/g liver) in 8-week-fed rats. In addition, compared with hepatic free fatty acid levels in rats fed the SD diet for 8 weeks and in rats fed the HF diet for 4 weeks (18.5 $\pm$ 0.6 μEq FFA/g liver), intake of the HF diet for 8 weeks resulted in significantly higher accumulation of hepatic free fatty acid and supplementing inulin to the HF diet reduced the accumulation of hepatic free fatty acids in 8-week-fed rats (Table 1). These

observations indicate that inulin intake suppressed the further development of hepatic steatosis. In addition, when we measured serum levels of insulin and β-hydroxybutyrate in 8-week-fed rats, there was no significant change in both levels in the 4 diet groups (data not shown). We interpret these data as indicating that feeding the HF diet for a long period of 8 weeks produced severe hepatic steatosis but did not induce diabetes.

Histological analysis of livers with HE and Oil Red O staining showed that steatosis was significantly improved after HF diet feeding for 8 weeks. As shown in Fig. 1A and 1B, lipid droplets accumulated in the cytoplasm of hepatocytes in both periportal and pericentral areas in the HF group. In comparison, lipid droplet accumulation in hepatocytes was significantly reduced in the HF+I group.

Dietary inulin ameliorates drug-induced liver injury in rats with fatty liver.

PB administration has been reported to induce oxidative changes in rat liver, detected as elevated hydroxyl radical levels (Kinoshita et al., 2002). We compared drug-induced liver injury in rats fed the SD and HF diets. Treatment of 8-week-fed rats with PB at a dose of 80 mg/kg once daily for 4 days resulted in increased liver weights and liver-to–body weight ratios in the SD, SD+I, HF and HF+I groups, and increased hepatic triacylglycerol levels in the HF and HF+I groups (Table 1). Staining of HF-fed rat liver with Oil Red O confirmed the lipid content of clear macrovacuoles (Fig. 1B), but there were few areas in the SD-fed rat liver stained with Oil Red O (data not shown). In addition to the further development of hepatic steatosis, there were areas of mixed inflammatory cell infiltration and hepatocyte necrosis dissecting the liver parenchyma following PB treatment (Fig. 1A and 1B). Consistent with morphological

changes, PB treatment of HF-fed rats resulted in significant increases in serum AST and ALT levels, but slight increases in serum γ-GTP levels, compared with those in the SD group (Fig. 2). At this time, the liver injury in the HF group was not accompanied by high levels of serum and hepatic TNFα and interleukin 6 as there was no significant difference of the TNFα and interleukin 6 levels in the 4 diet groups (data not shown). Furthermore, treatment of rats with dexamethasone at a dose of 40 mg/kg once daily for 3 days (*p.o.*) resulted in significant increases in serum levels of AST and ALT in the SD, HF and HF+I groups (Fig. 2). In contrast, inulin intake reduced the levels of serum AST and ALT in the HF group near to the levels in the SD group (Fig. 2). Correspondingly, hepatocyte necrosis was significantly attenuated in rats fed an inulin-supplemented HF diet after PB treatment (Fig. 1).

To examine whether the development of hepatic steatosis was associated with the setting of PB-induced liver injury, we compared serum AST and ALT levels following PB treatment in rats fed the SD and HF diets for 4 and 8 weeks. Figure 3 shows that treatment of 8-week-fed rats with PB at a dose of 80 mg/kg once daily for 4 days caused elevated levels of AST and ALT, but treatment of 4-week-fed rats with PB did not elevate the levels. These results suggest that hepatic steatosis further developed by feeding the HF diet for 8 weeks may be given to induce PB- and DEX-induced liver injury, and that inulin intake may ameliorate PB- and DEX-induced liver injury by reducing accumulated lipids such as triacylglycerols and free fatty acids in rat hepatocytes.

Next, to assess oxidative changes induced by PB administration in rat liver, we measured thiobarbituric acid-reactive substances (TBARS). As indicated by the accumulation of TBARS, the amount of total lipid peroxides was slightly higher in the

livers of rats fed the HF diet and administered PB than in the livers of rats fed the inulin-supplemented HF diet and administered PB (Fig. 4). In contrast, there was no significant change between hepatic TBARS levels in rats fed SD and SD+I diets (Fig. 4). These observations indicate that lowered lipid accumulation by inulin intake may be associated with the reduction of lipid peroxides produced by PB administration in the HF diet group (Fig. 4).

Hepatic expression and induction by PB of enzyme activities, mRNAs and proteins of CYPs in rats fed the 4 diets.

Since PB particularly induces CYP2B1/2B2 expression in rat liver, we first compared the enzyme activity of pentoxyresorufin *O*-dealkylation (PROD activities) in the 4 diet groups. As shown in Table 1, the HF and HF+I diets did not influence baseline or induced levels of microsomal protein. While the HF diet did not influence the baseline and induced levels of PROD activity, lower PROD activity in the HF+I group than in the SD group following PB treatment was detected (Table 1).

To assess the effects of the SD, SD+I, HF and HF+I diets on the hepatic expression of baseline and PB-treated CYP mRNAs, we measured hepatic mRNA levels in rats fed the 4 diets with and without PB treatment. Unexpectedly, fatty liver produced by feeding the HF diet down-regulated CYP2E1 mRNA expression to 69 $\pm$ 2% of the control (Table 2). The reduced levels were continued even after PB treatment for 4 days. Supplementing inulin to the HF diet did not reverse the mRNA levels of CYP2E1 (Table 2). In addition, there was no significant increase in the levels of CYP4A1, CYP4A2 and CYP4A3 in the vehicle- and PB-treated HF and HF+I groups.

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As shown in Table 2, there was no significant change in baseline mRNA levels of CYP2B1/2B2, CYP2C11, CYP3A1/3A2 and NADPH P450 reductase in the 4 diet groups. As expected, PB treatment markedly increased CYP2B1/2B2 mRNA levels in the 4 diet groups. PB-induced mRNA levels of CYP2B1 and CYP2B2 in the HF+I group were significantly lower than the SD group. Whereas there was no significant change in PB-induced mRNA levels of CYP2B1 and CYP2B2 between the SD and SD+I groups, PB-induced expression of CYP2B1 mRNA in the HF+I group was significantly declined compared with that in the HF group. PB-induced mRNA levels of CYP3A1 in the HF group were decreased compared with those in the SD group, and mRNA levels in the HF+I group were further decreased compared with those in the HF group. There was no significant difference in PB-induced mRNA levels of CYP3A2 in the 4 diet groups. The mRNA levels of NADPH P450 reductase in the SD and SD+I groups were increased following PB treatment. Whereas there was no significant difference in PB-treated mRNA levels of NADPH P450 reductase in the SD and HF groups, the levels in the HF+I group were lower than those in the SD group.

Next, we compared protein levels of CYPs in the 4 diet groups. The baseline and treated protein levels of CYP4A and PB-treated levels of CYP2E1 were decreased in the HF and HF+I groups (Fig. 5). There was no significant change in the baseline protein levels of CYP2B, CYP2C, CYP3A and NADPH P450 reductase except decreased levels of CYP2B protein in the HF+I group. The protein levels of CYP2B in the HF and HF+I groups following PB treatment were decreased to 76 $\pm$ 2% and 56 $\pm$ 1% of those in the SD group, respectively (Fig. 5). In addition, CYP3A protein levels in the HF+I group after PB treatment was reduced to 79 $\pm$ 1% of those in the HF group. CYP2C11 protein levels in the SD and SD+I groups were

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elevated after PB treatment, but the levels in the HF and HF+I groups were not significantly influenced. In contrast, there was no significant increase in protein levels of CYP2E1, CYP4A and NADPH P450 reductase in the 4 diet groups following PB treatment.

Effect of PB treatment on hepatic expression of mRNAs and proteins of transcription factors in rats fed the 4 diets.

CAR and PXR function as a heterodimer with RXRα, and HNF4α function as a homodimer, regulating genes encoding CYP2B, CYP2C and CYP3A (Honkakoski et al., 1998; Lehmann et al., 1998; Bogan et al., 2000; Chen et al., 2005; Tirona et al., 2003). We next analyzed the effects of the SD, SD+I, HF, HF+I diets and PB treatment on the expression of CAR, PXR, RXRα and HNF4α mRNAs in the livers. There was no significant change in the baseline mRNA levels of CAR, PXR, and RXRα in the 4 diet groups except that the levels of CAR were slightly increased in the HF and HF+I groups (Table 2). The mRNA levels of CAR and PXR following PB treatment exhibited a trend toward reduced levels in the 4 diet groups. No significant difference was observed in the mRNA levels of RXRα after PB treatment in the 4 diet groups. The baseline mRNA levels of HNF4α in the HF and HF+I groups and the treated levels in the HF group exhibited a trend toward reduced levels, and PB-treated levels in the HF+I group were reduced and lower than those in the HF group.

We further investigated the effect of the diets and PB treatment on the protein levels of nuclear receptors in the liver nucleus. As shown in Fig. 6, the protein levels of CAR in the nucleus were increased after PB treatment, but no difference was observed between the levels of the HF and HF+I groups. No significant difference was found in

the baseline and PB-treated protein levels of PXR and RXRα in the 4 diet groups. In contrast, the baseline and PB-treated protein levels of HNF4α in the HF group were decreased to 86 ± 5% and 77 ± 7% of ones in the SD group respectively, and the baseline and PB-treated levels in the HF+I group were 78 ± 13% and 46 ± 5% of those in the SD+I group respectively. In addition, the treated protein levels in the HF+I group were significantly lower than in the HF group.

Transcriptional cofactor PGC1α is considered to regulate biological programs linked to energy homeostasis through specific interaction with a variety of transcription factors such as nuclear hormone receptors (Puigserver and Spiegelman, 2003). Here, we investigated whether the expression of hepatic PGC1α mRNA and protein is influenced by the 4 diets and PB treatment. As shown in Table 2, no significant change in the baseline and treated levels of the 4 diet groups was detected, whereas a trend toward reduced PGC1α mRNA levels following PB treatment was observed in the 4 diet groups. Correspondingly, the baseline and treated levels of PGC1α protein were not significantly changed in the liver nucleus of rats fed each of 4 diets except that PB-treated levels of PGC1α protein in the SD group were decreased (Fig. 6). These observations indicate that alterations in the PB induction of CYPs by feeding the HF and HF+I diets may not be attributed to reduced levels of PGC1α protein in the nucleus of hepatocytes.

Discussion

This study demonstrates that feeding the high-lipid and high-sucrose diet for 8 weeks produces severe hepatic steatosis, which is associated with hepatic injury produced by the administration of xenobiotics such as PB and DEX (Table 1 and Figs. 1 and 2). Although large amounts of lipid deposition were evident in the liver sections of the rats, there was no inflammation and fibrosis in the liver of control rats (Fig. 1). This model is not an experimental model of nonalcoholic steatohepatitis, but is beneficial for investigating the effects of lipid accumulation on xenobiotics-induced liver injury. Liver injury by PB treatment was found in the HF group of 8-week-fed rats but not 4-week-fed rats (Fig. 3), consistent with the accumulation of hepatic triacylglycerols in the HF group of 8-week-fed rats being markedly higher than that of 4-week-fed rats. As found in liver sections (Fig. 1) and serum levels of AST and ALT (Fig. 2), inulin intake attenuated lipid droplets and PB- and DEX-induced hepatotoxic injury. These results suggest that (1) marked lipid accumulation in hepatocytes may be associated with the induction of liver injury by xenobiotics and (2) potential preventive effects of dietary inulin on blood glucose and lipid profiles, and hepatic lipid profiles may lead to the prevention of liver injury produced by xenobiotics. Steatosis produced by diets deficient in lipotropes such as choline and methionine (Weltman et al., 1996) and that induced by chemicals such as orotic acid (Su et al., 1999) are established as experimental models of nonalcoholic steatohepatitis and drug-mediated liver injury, respectively. Those models have been reported to be associated with liver injuries via the alteration of hepatic expression of drug-metabolizing enzymes such as CYP2E1, CYP4A, and CYP2C11. In addition, a pathophysiological status such as chemically induced and spontaneous diabetes has been demonstrated to influence the hepatic

expression of CYP2B, CYP2C11, CYP3A and CYP4A (Dong et al., 1988; Favreau and Schenkman, 1988; Bellward et al., 1988; Woodcroft et al., 1999). Nevertheless, the detailed effects of lipid accumulation on the expression of transcriptional factors, which regulate the gene expression of drug-metabolizing enzymes, remain to be clarified. This study establishes that dietary manipulation such as high lard and high sucrose produces hepatic steatosis associated with xenobiotics-induced liver injury (Figs. 1 and 2 and Table 1). Thus, in this study, we examined the effects of lipid accumulation on baseline and xenobiotics-treated expression of CYP2E1, CYP2B, CYP2C11, CYP3A and CYP4A mRNAs and proteins and the association between alterations in the expression of CYPs and transcriptin factors and xenobiotics-induced liver injury.

Leclercq et al. (2000) have demonstrated that CYP2E1 in wild-type mice but CYP4A in *CYP2e1*$^{-/-}$ mice is up-regulated in nonalcoholic steatohepatitis and plays a key role in the development of liver injury by initiating lipid peroxidation. In contrast, feeding a high-fat and high-sucrose diet down-regulated CYP2E1 and CYP4As mRNAs and proteins, and even after treatment with PB, the enzymes remained to be down-regulated (Table 2 and Fig. 5). These results indicate that CYP2E1 and CYP4As seemed not to play a central role in this PB-induced liver injury.

Rats fed the HF+I diet exhibited reduced induction in response to PB treatment as evidenced by PROD activities and CYP2B mRNA and protein levels (Tables 1 and 2 and Fig. 5). Zannikos et al. (1993) have demonstrated that rats fed the energy-dense diet for 32 weeks as compared with rats fed the control diet exhibit a lower increase in PROD activities following PB treatment (20-fold increase *vs.* 45-fold increase), while similar amounts of protein and mRNA of CYP2B1/2B2 are found. Hepatic nutritional status in this experimental model appears to be rather different from the energy-dense

diet model; lipid accumulation in the liver of rats fed the HF diet for 8 weeks was over 2-fold higher than that of rats fed the energy-dense diet for 32 weeks. As pointed out by Zannikos et al. the change in the microsomal lipid environment based on diet and/or aging may influence enzyme activity.

PB activates CAR and PXR that bind to and transactivate CYP2B and CYP3A promoters (Honkakoski et al., 1998; Lehmann et al., 1998). HNF4α synergistically enhanced CAR- and PXR-mediated xenobiotic induction of human CYP2C9 (Chen et al., 2005) and CYP3A (Tirona et al., 2003). Although there is no direct evidence that HNF4α synergistically enhances the CAR- and PXR-mediated xenobiotic induction of CYP2B, the CYP2B2 phenobarbital response unit contains an HNF4 binding site (Beaudet et al., 2005). In addition, single nucleotide polymorphism in a putative HNF4 binding site in the phenobarbital response element of CYP2B6 has been demonstrated to be correlated with lowered CYP2B6 expression (Lamba et al., 2003). By a study using HNF4 antisense RNA it has been demonstrated that HNF4 is a general regulator of major drug-metabolizing CYPs such as CYP3A4, CYP3A5, CYP2A6, CYP2B6, CYP2C9 and CYP2D6 (Jover et al., 2001). Thus, in this study, we assessed whether the reduced PB induction of CYP2B and CYP3A mRNAs and proteins in the HF+I group may be attributable to a decline in the expression of transcription factors. While PGC1α has been demonstrated to mediate the ligand-independent activation of CAR (Shiraki et al., 2003), the levels of PGC1α mRNA and protein in the HF+I group were similar to the control of the SD+I and HF groups (Table 2 and Fig. 6), suggesting that PGC1α may not play a central role in the preventive action of dietary inulin. There was no significant change based on HF and HF+I diets in the baseline and PB-treated expression of CAR, PXR, and RXRα mRNAs and proteins except increased

levels of CAR mRNA in the vehicle-treated HF+I group (Table 2 and Fig. 6). In contrast, HNF4α protein significantly declined in the liver nucleus of rats fed the HF and HF+I diets (Fig. 6). Thus the reduced induction of hepatic CYP2B and CYP3A in PB-treated HF+I group may be attributable to a decline in the expression of HNF4α protein in the liver nucleus. Serum glucose levels were increased in HF-fed and vehicle-treated rats and inulin-supplemented diet suppressed the increase (Table 1). Furthermore, the levels in HF-fed rats after PB treatment exhibited a tendency to remain increased. Glucose itself has been demonstrated to induce the expression of HNF4α mRNA in primary-cultured rat hepatocytes (Oyadomari et al., 2000). Thus, the lower levels of HNF4α mRNA and protein in the PB-treated HF+I group than those in the HF group may be associated with suppression of increase in serum glucose by inulin. Dhe-Paganon et al. presume that fatty acids or related molecules might be the endogenous ligands for HNF4α, that is, HNF4α may function as a biosensor for fatty acids within the cell (2002). However, it remains to be addressed whether the declined expression of HNF4α protein is influenced by liver lipid environment, that is, marked accumulation of liver fatty acids as found in the HF and HF+I groups. Analysis of the HNF4α gene expression is currently progressing in our laboratory.

We proposed the scheme of PB-induced liver injury associated with lipid accumulation and the suppression by inulin. Absorption of a high-fat and high-sucrose diet was concomitant with increases in blood glucose and liver lipids (Table 1). Glucose has been demonstrated to induce transciption factors such as sterol regulatory element binding protein-1c, which enhances transcription of genes involved in de novo lipogenesis such as fatty acid synthase, leading to increased syntheses of fatty acids and triacylglycerols. Kinoshita et al. (2002) have demonstrated that a significant increase

of hydroxy radical levels by day 4 of high-dose treatment with PB accompanied the accumulation of 8-hydroxydeoxyguanosine in the nucleus. In fact, as indicated by the accumulation of TBARS, lipid peroxides were slightly increased in the HF groups after PB treatment (Fig. 4). It is possible that CYP2B and CYP3A induced by PB exposure may contribute to produce reactive oxygen species or that changed microsomal lipid environment may prevent the elimination of oxygen radicals leading to lipid peroxidation. On the other hand, Zhang et al. (2002) have demonstrated that overdoses of acetaminophen cause significant hepatotoxicity via a pathway mediated by CAR, because PB increases susceptibility to acetaminophen damage in wild-type but not in CAR null mice. Furthermore, because PXR (-/-) mice are less sensitive to acetoaminophen hepatotoxicity, PXR appears to be an important modulator (Wolf et al., 2005). In addition, Dai et al. (2005) have presented that RXRα regulates the expression of glutathione S-transferase genes and modulates acetoaminophen-glutathione conjugation in mouse liver, because RXRα null mice are protected from acetoaminophen-induced hepatotoxicity and exhibit greater levels of acetoaminophen-glutathione in the liver. Thus, a potential hepatotoxic compound in fatty liver may cause acute liver failure following PB treatment via a CAR and/or PXR-mediated pathway or associated with an RXRα-glutathione *S*-transferase regulatory network (Fig. 7). Together, we speculate that inulin suppresses increase in blood glucose, that leads to a coordinate suppression of lipogenic gene transcription causing in turn a decrease in excessive accumulation of liver lipids, and reduces expression of HNF4α causing a decrease in xenobitic-induced expression of CYP2B and CYP3A. The model may explain the inhibitory effect of inulin on xenobiotic-induced liver failure.

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Footnote

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Legends for scheme and figures

Fig. 1 Histologic analysis of liver from rats fed SD, SD+I, HF, HF+I for 8 weeks and then administered PB (80mg/kg, *i.p.*) or saline as vehicle once daily for 4 days. The livers were stained with HE (magnification, x 200) (A) and Oil Red O (magnification, x 200) (B). Blurred trabecular structure of the liver lobule with distinctly enlarged clear and vacuolated hepatocytes and numerous oil red O positive hepatocytes can be seen throughout the hepatic lobule in rats fed HF. The lower intensity of oil red O staining can be seen in rats fed HF+I. Mixed inflammatory infiltrate with lymphocyte and polymorphonuclear neutrophil necroinflammation is dispersed in the liver lobule of rats administered PB, but rare necroinflammation can be seen in the liver lobule of rats administered vehicle. Scale bar indicates 100 μm.

Fig. 2 Inulin-supplemented diet normalizes serum AST and ALT activities in rats administered PB (80 mg/kg, *i.p.*) or DEX (50mg/kg, *p.o.*). The rats were then sacrificed, and serum AST (A, D), ALT (B, E) and γ-GTP levels were measured. Values are the means ± SE for 6 to 7 determinants in each group. **p<0.01, ***p<0.001 for HF- or HF+I-fed and PB-administered animals versus SD-fed and PB-administered animals; ###p<0.001 for HF+I-fed and PB-administered animals versus HF-fed and PB-administered animals.

Fig. 3 Effect of phenobarbital treatment on serum levels of AST and ALT in rats fed SD and HF for 4 and 8 weeks. Values are the means ± SE of 6-7 determinants. ***p<0.001 for HF-fed and PB-administered animals versus SD-fed and PB-administered animals; +++p<0.001 for PB-administered animals versus vehicle-administered animals.

Fig. 4 Inulin down-regulates thiobarbituric acid-reactive substance (TBARS) levels in the livers of rats fed HF for 8 weeks and then administered PB once daily for 4 days. Values are the means ± 7 determinations. *p<0.05 for HF-fed and vehicle-administered animals versus SD-fed and vehicle-administered animals or HF-fed and PB-administered animals versus SD-fed and PB-administered animals; +p<0.05, ++p<0.01, +++p<0.001 for for PB-administered animals versus vehicle-administered animals; #p<0.05, ##p<0.01 for HF+I-fed and vehicle-administered animals versus HF-fed and vehicle-administered animals or HF+I-fed and PB-administered animals versus HF-fed and PB-administered animals. A, TBARS (nmol/mg protein); B, TBARS (nmol/liver).

Fig. 5 Effects of phenobarbital treatment on CYP protein levels in rats fed SD, SD+I, HF, and HF+I for 8 weeks. Rats were administered PB (80 mg/kg, *i.p.*) once daily for 4 days and then sacrificed, and the microsome proteins (20 μg/lane) were prepared and subjected to immunoblot analysis. The signal intensities were determined with a Fujix BAS-2000 bioimage analyzer (Fuji Photo Film). Relative levels are expressed by taking the control values obtained from SD-fed and vehicle-treated animals as 100. *p<0.05, **p<0.01, ***p<0.001 for SD+I-, HF-, or HF+I-fed and vehicle-administered animals versus SD-fed and vehicle-administered animals or SD+I-, HF-, or HF+I-fed and PB-administered animals versus SD-fed and PB-administered animals; ++p<0.01, +++p<0.001 for PB-administered animals versus vehicle-administered animals; ##p<0.01, ###p<0.001 for HF+I-fed and PB-administered animals versus HF-fed and PB-administered animals.

Fig. 6 Effects of phenobarbital treatment on nuclear receptor protein levels in rats fed

SD, SD+I, HF, and HF+I for 8 weeks. Rats were fed the 4 diets for 8 weeks and then administered PB (80 mg/kg, *i.p.*) once daily for 4 days, and then sacrificed. The nuclear proteins (20 μg/lane) were prepared and subjected to immunoblot analysis. The signal intensities were determined with a Fujix BAS-2000 bioimage analyzer (Fuji Photo Film). Relative levels are expressed by taking the control values obtained from SD-fed and vehicle-treated animals as 100. *p<0.05, **p<0.01, ***p<0.001 for SD+I-, HF-, or HF+I-fed and vehicle-administered animals versus SD-fed and vehicle-administered animals or SD+I-, HF-, or HF+I-fed and PB-administered animals versus SD-fed and PB-administered animals; +p<0.05, +++p<0.001 for PB-administered animals versus vehicle-administered animals; ##p<0.01 for HF+I-fed and PB-administered animals versus HF-fed and PB-administered animals.

Fig. 7 We proposed a scheme of PB-induced liver injury associated with lipid accumulation and the suppression by inulin. See text for explanations. GST, glutathione *S* transferase; FFA, free fatty acid; TG, triacylglycerol.

Supplemental data of Figure 5

Effects of phenobarbital treatment on CYP protein levels in rats fed SD, SD+I, HF, and HF+I for 8 weeks. Rats were administered PB (80 mg/kg, *i.p.*) once daily for 4 days and then sacrificed, and the microsome proteins (20 μg/lane) were prepared and subjected to immunoblot analysis.

Supplemental data of Figure 6

Effects of phenobarbital treatment on nuclear receptor protein levels in rats fed SD, SD+I, HF, and HF+I for 8 weeks. Rats were fed the 4 diets for 8 weeks

and then administered PB (80 mg/kg, *i.p.*) once daily for 4 days, and then sacrificed. The nuclear proteins (20 μg/lane) were prepared and subjected to immunoblot analysis.

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Table 1. Effect of phenobarbital treatment on body weight, liver weight, liver lipids, serum lipids, serum glucose and microsomal enzyme activity in SD-, SD+I-, HF-, and HF+I-fed rats for 8 weeks and then administered PB.

	Treatment	SD	SD + I	HF	HF + I
Body weight (g)	C	289.7 ± 5.3	279.2 ± 8.3	287.1 ± 4.4	258.5 ± 4.9$^{**,\#}$
	PB	285.9 ± 7.0	272.9 ± 6.6	281.6 ± 7.2	256.5 ± 7.9$^{**,\#}$
Liver weight (g)	C	9.80 ± 0.22	9.27 ± 0.23	14.07 ± 0.33***	12.51 ± 0.39$^{***,\#\#}$
	PB	11.63 ± 0.15^{+++}	11.55 ± 0.42^{+++}	16.53 ± 0.63$^{***,+++}$	15.14 ± 0.42$^{***,+++}$
Ratio (Liver weight/ body weight)	C	0.032 ± 0.001	0.034 ± 0.001	0.051 ± 0.002	0.047 ± 0.002
	PB	0.040 ± 0.001^{+++}	0.042 ± 0.001^{++}	0.059 ± 0.001^{+++}	0.059 ± 0.003^{+++}
Liver triacylglycerol (mg/g liver)	C	12.93 ± 0.82	14.27 ± 0.34	72.55 ± 8.67***	44.80 ± 5.18$^{***,\#}$
	PB	21.25 ± 2.62	13.89 ± 0.43	103.57 ± 6.48$^{***,+++}$	94.29 ± 6.63$^{***,+++}$
Liver total choresterol (mg/g liver)	C	4.73 ± 0.92	3.29 ± 0.49	32.47 ± 3.74***	27.88 ± 5.52***
	PB	5.95 ± 0.82	4.09 ± 0.70	26.78 ± 3.12***	32.21 ± 7.06***
Liver free fatty acid (μEq/g liver)	C	8.97 ± 0.58	8.89 ± 0.69	49.86 ± 2.55***	43.76 ± 2.46$^{***,\#}$
	PB	11.08 ± 1.02	9.22 ± 0.48	36.50 ± 3.62$^{***,+++}$	41.33 ± 4.83$^{***,+++}$
Serum triacylglycerol (g/L)	C	1.54 ± 0.12	1.47 ± 0.17	1.73 ± 0.25	1.11 ± 0.10$^{\#}$
	PB	0.94 ± 0.07	0.91 ± 0.05	2.10 ± 0.28***	1.83 ± 0.25**
Serum total cholesterol (g/L)	C	0.60 ± 0.02	0.57 ± 0.02	2.17 ± 0.29***	2.21 ± 0.17***
	PB	0.90 ± 0.02	0.82 ± 0.04	1.78 ± 0.01***	1.92 ± 0.20***
Serum free fatty acid (μEq/L)	C	462.6 ± 19.3	454.1 ± 33.4	831.1 ± 93.9***	651.9 ± 84.1$^{*,\#}$
	PB	451.3 ± 36.3	462.5 ± 40.7	905.5 ± 67.8***	827.6 ± 81.1***
Serum glucose (g/L)	C	1.34 ± 0.07	1.34 ± 0.09	1.60 ± 0.11*	1.35 ± 0.05$^{\#}$
	PB	1.21 ± 0.08	1.26 ± 0.04	1.41 ± 0.11	1.25 ± 0.07
Microsome protein (mg/g liver)	C	25.4 ± 1.3	22.5 ± 1.6	26.9 ± 1.1	26.0 ± 1.0
	PB	24.7 ± 1.5	23.7 ± 0.8	25.9 ± 2.7	27.8 ± 0.7
Pentoxyresorufin *O*-dealkylation (pmol/mg microsome rotein/min)	C	10.0 ± 1.9	12.7 ± 2.3	8.0 ± 1.2	7.1 ± 0.6
	PB	813.5 ± 51.5^{+++}	786.8 ± 81.6^{+++}	751.5 ± 61.3^{+++}	640.6 ± 49.8$^{*,+++}$

Values are means ± SE of 6-7 determinants. C, vehicle; PB, phenobarbital. *p<0.05, **p<0.01, ***p<0.001 for SD+I-, HF-, or HF+I-fed and vehicle-administered animals versus SD-fed and vehicle-administered animals, or SD+I-, HF-, or HF+I-fed and PB-administered animals versus SD-fed and PB-administered animals; ++p<0.01, +++p<0.001 for PB-administered animals versus vehicle-administered animals; #p< 0.05, ##p<0.01 for HF+I-fed and vehicle-administered animals versus HF-fed and vehicle-administered animals or HF+I-fed and PB-administered animals versus HF-fed and PB-administered animals.

Table 2. Liver gene expression in rats fed SD-, SD+I-, HF-, or HF+I for 8 weeks and administered PB (80 mg/kg, *i.p.*) once daily for 4 days.

Gene	Accession number	SD (Fold induction)		SD + I (Fold induction)		HF (Fold induction)		HF + I (Fold induction)	
		Vehicle	PB	Vehicle	PB	Vehicle	PB	Vehicle	PB
CYP2B1	J00719	1.00 ± 0.19	331.21 ± 21.89^{+++}	1.12 ± 0.58	318.29 ± 39.40^{+++}	1.08 ± 0.42	288.76 ± 20.40^{+++}	2.07 ± 1.41	225.16 ± 9.57$^{**,+++,\#}$
CYP2B2	M34452	1.00 ± 0.17	75.03 ± 3.78^{+++}	0.92 ± 0.17	71.39 ± 11.33^{+++}	0.82 ± 0.10	44.80 ± 7.03$^{***,+++}$	0.98 ± 0.22	39.71 ± 4.55$^{***,+++}$
CYP2C11	NM_019184	1.00 ± 0.12	1.49 ± 0.43	0.79 ± 0.14	2.80 ± 0.05	2.21 ± 0.62	2.92 ± 1.38	6.50 ± 4.86	3.46 ± 1.89
CYP2E1	NM_031543	1.00 ± 0.19	0.76 ± 0.05	0.96 ± 0.03	0.66 ± 0.08^{+}	0.69 ± 0.02*	0.37 ± 0.02$^{**,+}$	0.57 ± 0.03**	0.32 ± 0.07$^{**,+}$
CYP3A1	NM_173144	1.00 ± 0.05	15.47 ± 0.92^{+++}	0.92 ± 0.09	10.55 ± 1.78^{+++}	1.14 ± 0.17	7.67 ± 1.26$^{***,+++}$	1.01 ± 0.30	5.34 ± 0.46$^{***,+++,\#}$
CYP3A2	NM_153312	1.00 ± 0.38	9.99 ± 3.22^{+++}	0.43 ± 0.05	6.73 ± 1.73^{+}	0.99 ± 0.22	5.45 ± 2.22	1.24 ± 0.08	4.26 ± 0.36
CYP4A1	M57718	1.00 ± 0.09	0.88 ± 0.09	1.24 ± 0.23	0.75 ± 0.04^{+}	0.71 ± 0.09	0.52 ± 0.09*	0.54 ± 0.06	0.75 ± 0.18
CYP4A2	M57719	1.00 ± 0.04	1.96 ± 0.48^{+}	1.75 ± 0.67	1.07 ± 0.47	0.58 ± 0.09	0.53 ± 0.09**	0.38 ± 0.03	0.37 ± 0.03**
CYP4A3	M33936	1.00 ± 0.14	1.58 ± 0.23	1.09 ± 0.16	1.11 ± 0.20	0.96 ± 0.12	1.09 ± 0.12	0.92 ± 0.01	0.63 ± 0.01$^{**,\#}$
NADPH cytochrome P450 reductase	NM_031576	1.00 + 0.09	1.82 + 0.12^{+}	1.00 + 0.07	2.44 + 0.41^{+++}	0.95 + 0.22	1.57 + 0.22	0.84 + 0.20	1.07 + 0.25*
CAR	NM_022941	1.00 ± 0.10	0.51 ± 0.09	0.71 ± 0.20	0.36 ± 0.07	1.80 ± 0.21	0.76 ± 0.24	2.32 ± 0.89*	0.57 ± 0.17
PXR	NM_052980	1.00 ± 0.09	0.54 ± 0.09	0.83 ± 0.07	0.45 ± 0.03	0.84 ± 0.15	0.64 ± 0.21	0.81 ± 0.29	0.36 ± 0.08
RXRα	NM_012805	1.00 ± 0.08	1.30 ± 0.11	1.48 ± 0.10	1.38 ± 0.10	1.23 ± 0.20	1.19 ± 0.10	1.01 ± 0.06	0.83 ± 0.14
HNF4α	NM_022180	1.00 ± 0.06	1.04 ± 0.13	0.97 ± 0.03	1.04 ± 0.07	0.86 ± 0.05	0.77 ± 0.07	0.76 ± 0.13	0.48 ± 0.05$^{**,\#}$
PGC-1α	AY237127	1.00 ± 0.40	0.43 ± 0.07	0.94 ± 0.48	0.69 ± 0.25	0.70 ± 0.30	0.69 ± 0.16	0.88 ± 0.18	0.46 ± 0.17

Values are means ± SE of 3-5 determinations; *p<0.05, **p<0.01, ***p<0.001 for SD+I-, HF- or HF+I-fed and vehicle-administered animals versus SD-fed and vehicle-administered animals or SD+I-, HF- or HF+I-fed and PB-administered animals versus SD-fed and PB-administered animals; +p<0.05, +++p<0.001 for PB-administered animals versus vehicle-administered animals; #p<0.05 for HF+I-fed and vehicle-administered animals versus HF-fed and vehicle-treated animals or HF+I-fed and PB-treated animals versus HF-fed and PB-treated animals.

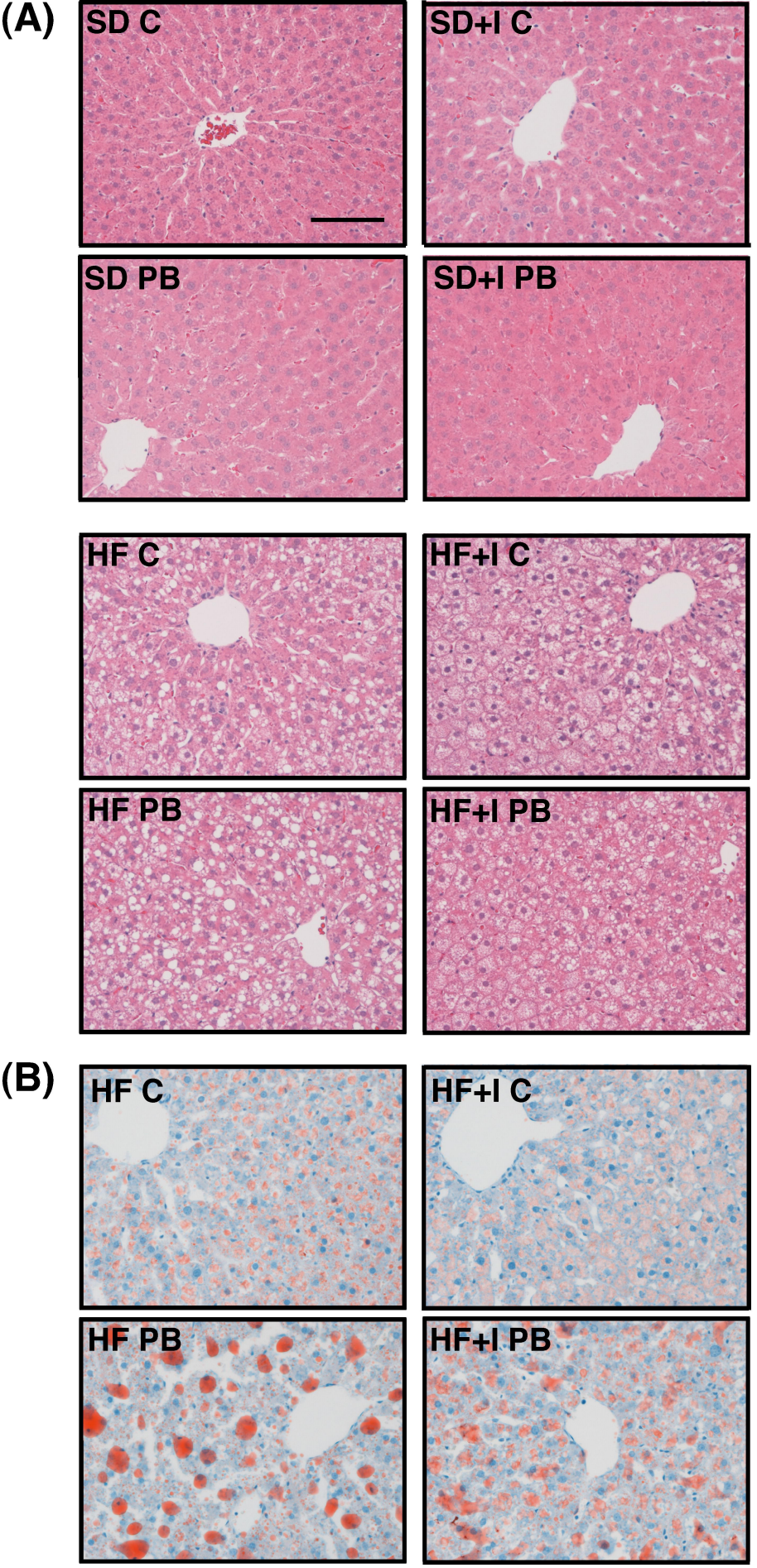
(A)
SD C
SD+I C
SD PB
SD+I PB
HF C
HF+I C
HF PB
HF+I PB
(B)
HF C
HF+I C
HF PB
HF+I PB

Fig. 1

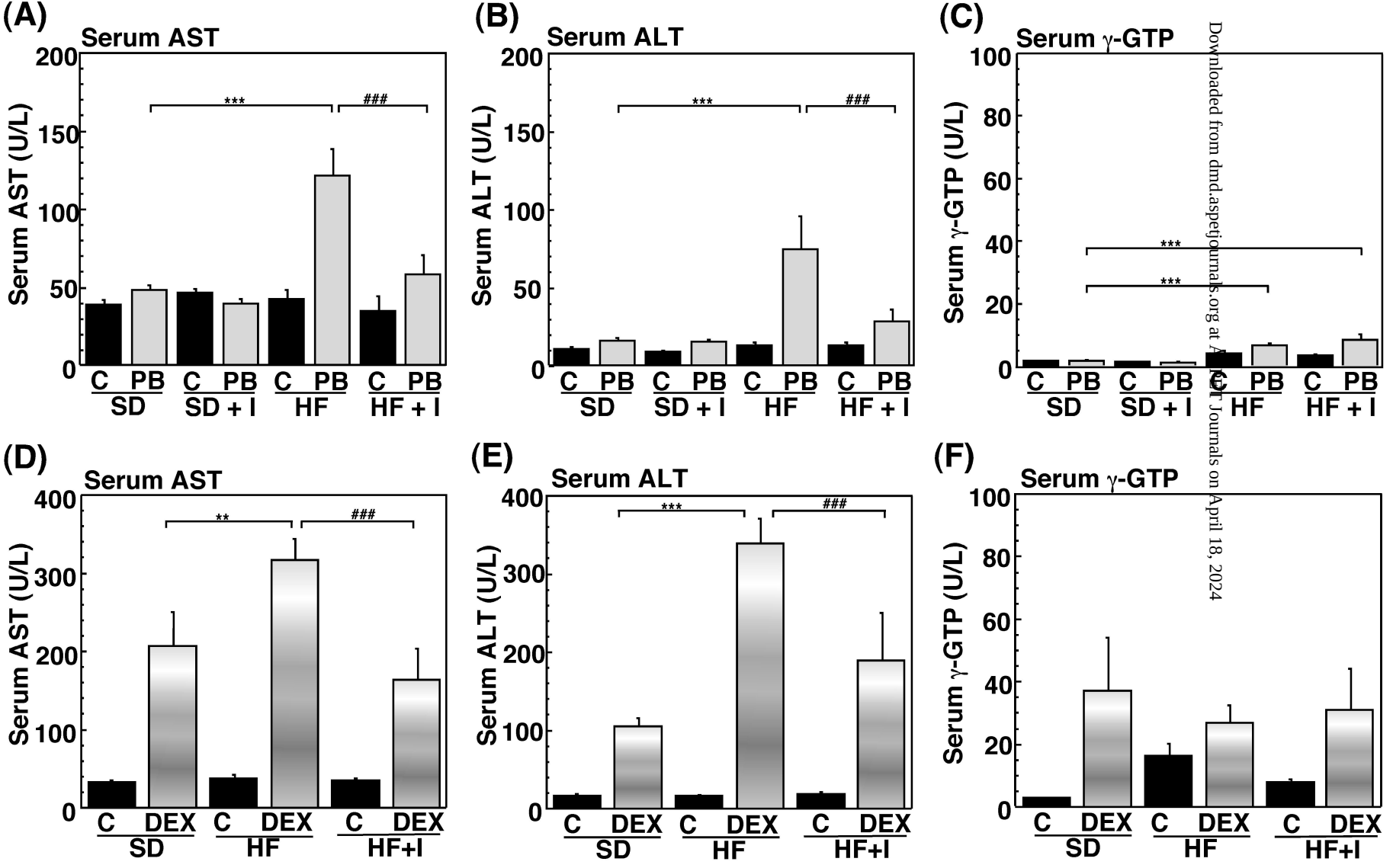

Fig. 2

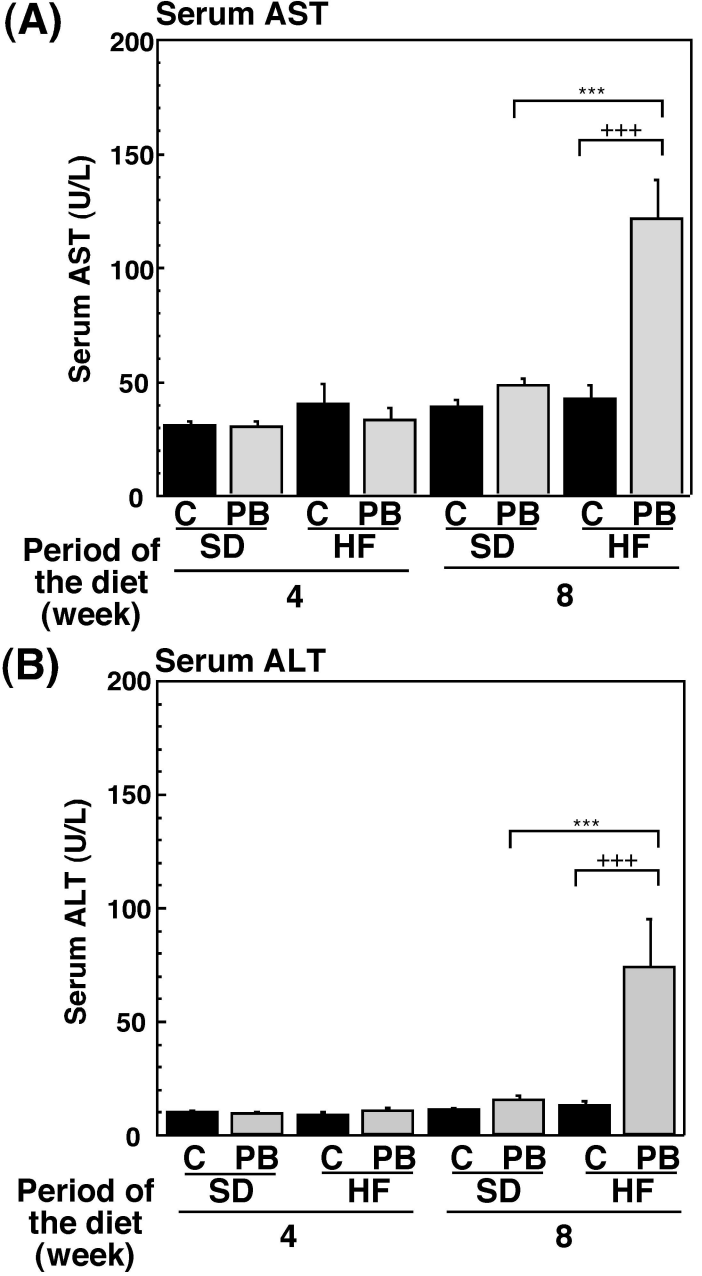
(A)
Serum AST
Serum AST (U/L)
200
150
100
50
0
***
+++
C PB C PB C PB C PB
SD HF SD HF
Period of the diet (week)
4
8
(B)
Serum ALT
Serum ALT (U/L)
200
150
100
50
0
***
+++
C PB C PB C PB C PB
SD HF SD HF
Period of the diet (week)
4
8

Fig. 3

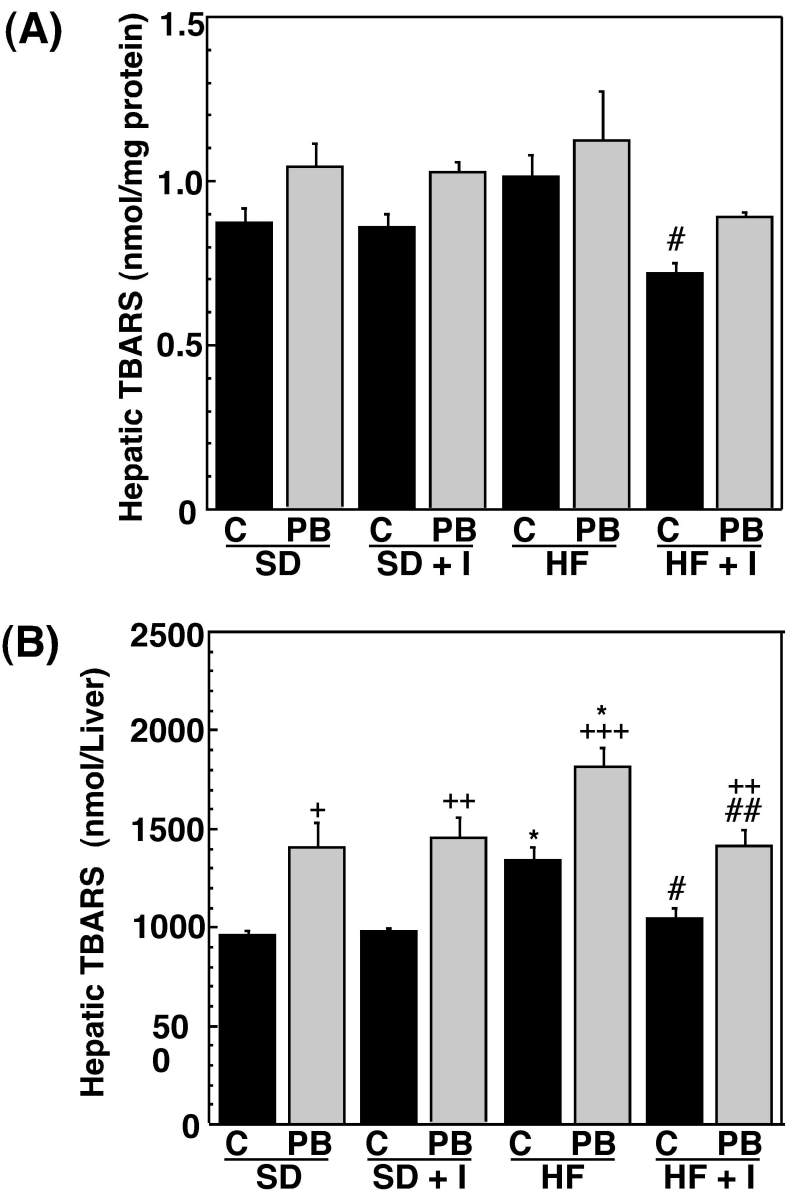
(A)
Hepatic TBARS (nmol/mg protein)
1.5
1.0
0.5
0
#
C PB C PB C PB C PB
SD SD + I HF HF + I
(B)
Hepatic TBARS (nmol/Liver)
2500
2000
1500
1000
50
0
0
+
++
*
+++
++
##
#
C PB C PB C PB C PB
SD SD + I HF HF + I

Fig. 4

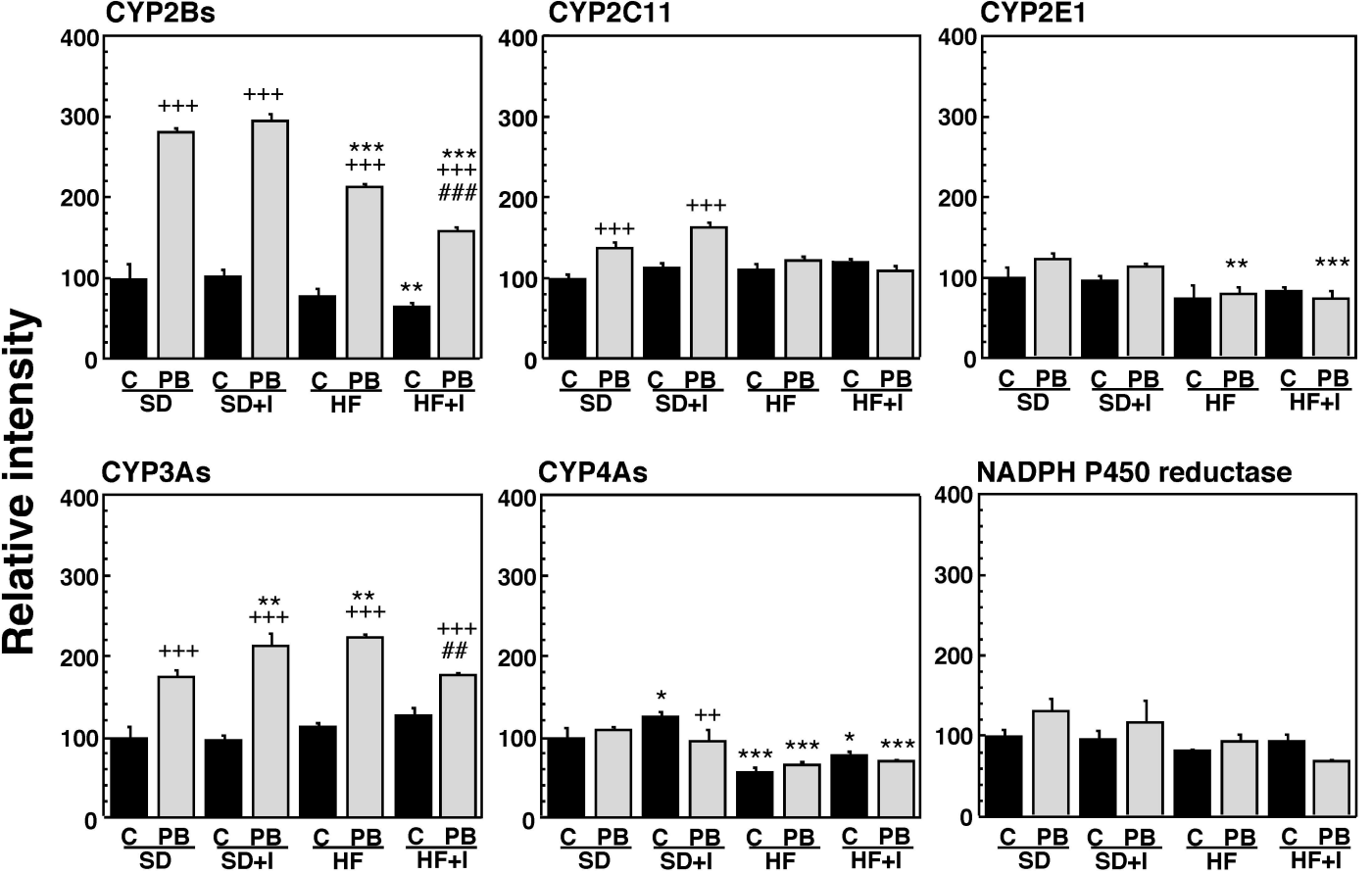
CYP2Bs
CYP2C11
CYP2E1
CYP3As
CYP4As
NADPH P450 reductase
Relative intensity
400
300
200
100
0
C PB
SD
SD+I
HF
HF+I
+++
***
**
*
++
###
##

Fig. 5

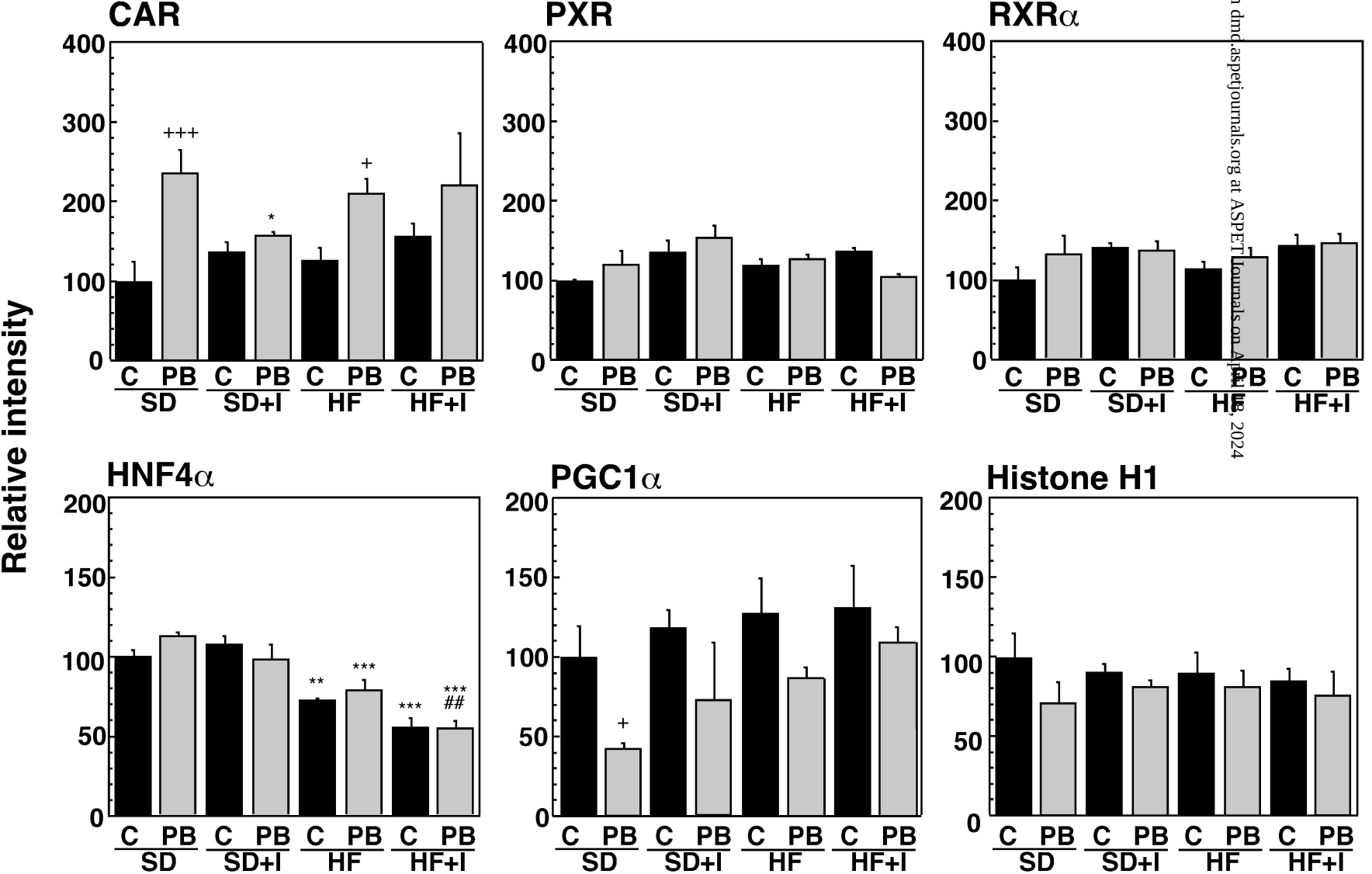

Fig.6

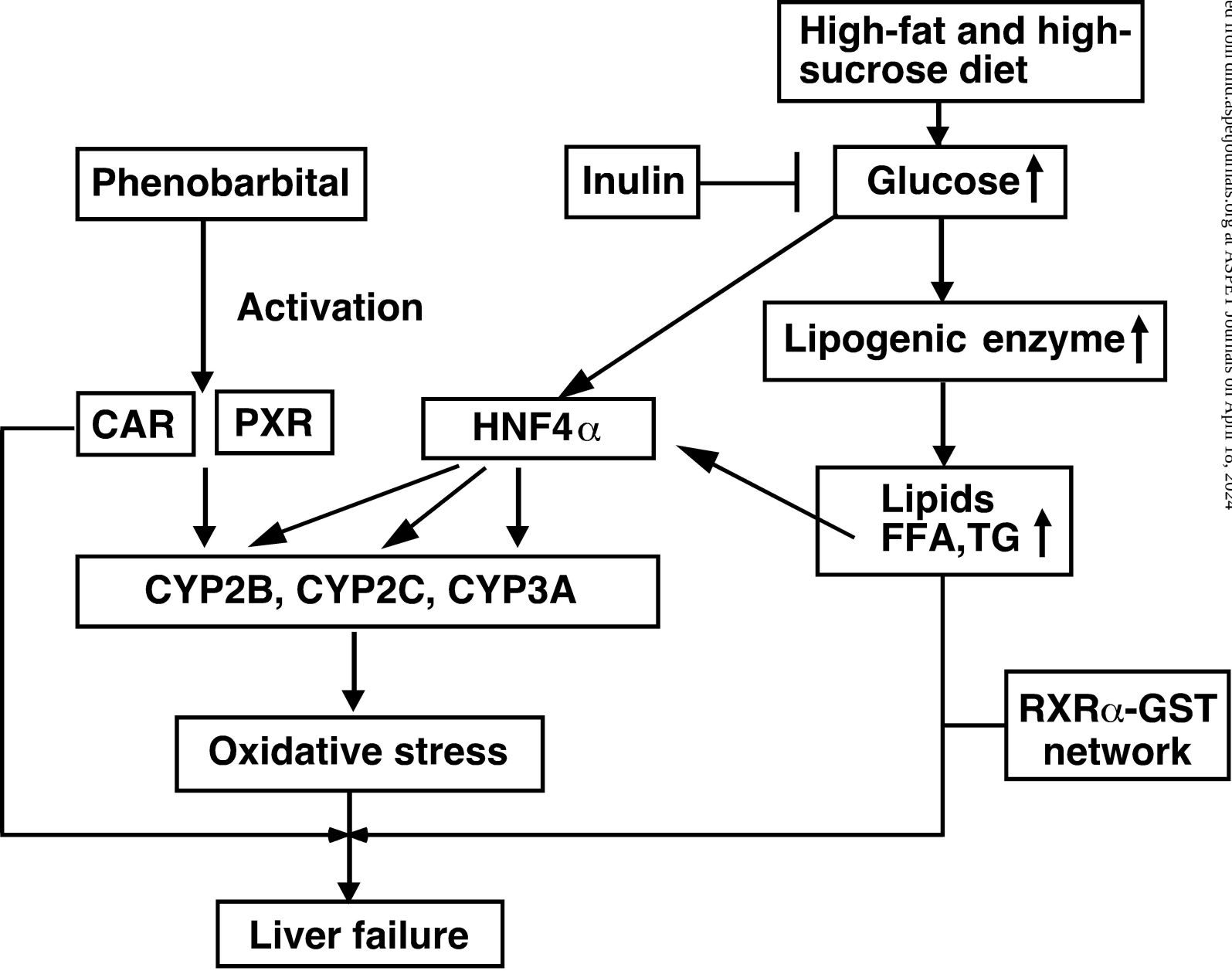

Fig.7