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# Expression of hepatic UDP-glucuronosyltransferase 1A1 and 1A6 correlated with increased expression of the nuclear constitutive androstane receptor and peroxisome proliferator-activated receptor α in male rats fed a high-fat and high-sucrose diet.

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B) ABBREVIATIONS: AG, acetaminophen glucuronide; AhR, aryl hydrocarbon receptor; APAP, acetaminophen; ARNT, AhR nuclear translocator; AUC, area under plasma concentration – time curve; CAR, constitutive androstane receptor; CFB, clofibrate; CPR, NADPH-cytochrome P450 reductase; HF1 diet, high-fat and high-sucrose diet; HF2 diet, high-fat diet; HNF, hepatocyte nuclear factor; Mrp, multidrug resistance- associated protein; NAPQI, *N*-acetyl-*p*-benzoquinoneimine; Nrf2, nuclear factor E2-related factor 2; Oatp, organic anion transporter polypeptide; P450, cytochrome P450; PB, phenobarbital; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1α; PH, 1,7-phenanthroline; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; PCR, polymerase chain reaction; RXR, retinoid X receptor ; SD diet, standard diet; UGT, UDP-glucuronosyltransferase; XRE, xenobiotic response element.

**Abstract**

Rats that consumed a high-fat and high-sucrose (HF1) diet or a high-fat (HF2) diet developed hepatic steatosis. The alteration in nutritional status affected hepatic cytochrome P450 and UDP-glucuronosyltransferase (UGT) levels. Messenger RNA and protein levels of UGT1A1 and UGT1A6 in the liver but not the jejunum were increased in male rats fed the HF1 diet. These protein levels did not increase in HF2-fed male rats or HF1-fed female rats. In contrast, the CYP1A2 protein level was decreased in the HF1 but not HF2 diet group, whereas CYP2E1 and CYP4A protein levels were elevated in the HF2 but not HF1 diet group. No significant difference in the Oatp1, Oatp2, Mrp2 or Mrp3 protein levels were found between the standard and HF1 diet groups of male rats. Consumption of the HF1 diet affected the in vivo metabolism of acetaminophen (APAP) such that the area under the APAP-glucuronide plasma concentration – time curve was elevated 2.1-fold in male rats but not female rats. In liver cell nuclei of male rats but not female rats, constitutive androstane receptor (CAR) and proliferator–activated receptor α (PPARα) protein levels were significantly enhanced by intake of the HF1 diet. Additionally, administration of the PPARα agonist clofibrate to male rats up-regulated UGT1A1 and UGT1A6 and down-regulated CYP1A2 in the liver. Taken together, these results indicate that nutritional status may gender-specifically influence the expression and activation of CAR and PPARα in liver cell nuclei, and this effect appears to be associated with alterations in UGT1A1 and UGT1A6 expression.

**Introduction**

Liver drug-metabolizing enzymes and drug transporters play a central role in the metabolism and elimination of therapeutic drugs and environmental contaminants. Information on the activities and expression of drug-metabolizing enzymes and drug transporters is essential for the development of customized medical treatments. The expression of drug-metabolizing enzymes and drug transporters in the body is affected by genetic factors and also by nongenetic factors such as environmental factors. Nutritional states such as starvation, fasting, and high-lipid diet, and pathophysiological alterations such as diabetes have been reported to modulate the liver drug-metabolizing phase I enzyme composition, leading to altered hepatic metabolism of drugs, carcinogens, steroid hormones, and fatty acids. The accumulation in the liver of triacylglycerols, defined as hepatic steatosis, is proposed to be the first stage for more severe liver diseases such as nonalcoholic steatohepatitis, which shows histologic signs of fibrosis and necroinflammation, through cirrhosis, terminal liver failure, and hepatocellular carcinoma (Bugianesi et al., 2002). The elevated expression of CYP2E1, a microsomal oxidase involved in fatty acid ω-oxidation, as well as CYP4A has been shown to be largely responsible for the pathogenesis of liver disease in patients with nonalcoholic steatohepatitis (Weltman et al., 1996; Emery et al., 2003; Chalasami et al., 2003). In a previous study, we demonstrated that feeding rats a high-lipid and high-sucrose diet (HF1 diet) produces hepatic steatosis, which is associated with the induction of liver injury by xenobiotics such as phenobarbital (PB) and dexamethasone, but not with liver injury via the alteration of hepatic CYP2E1 and CYP4A expression (Sugatani et al., 2006). A number of metabolic food-drug interactions involving drug-metabolizing phase I enzymes and drug transporters have been documented including the interaction of St. John's wort with cyclosporin and alterations in the expression of drug-metabolizing phase I enzymes and drug transporters dependent on nutritional states such as fasting and hepatic steatosis.

However, little is known about the effects of nutritional status on the expression of drug-metabolizing phase II enzymes, although there have been several reports of xenobiotic- and food component-induced expression (Shelby and Klaassen, 2006; Sugatani et al., 2004).

Acetaminophen (APAP) at therapeutic doses is mainly eliminated through glucuronidation and sulfation, although a small fraction is oxidized by CYP2E1, CYP3A and CYP1A2 to N-acetyl-*p*-benzoquinone-imine (NAPQI), a toxic metabolite. In this study, we therefore investigated whether elevated levels of UGT1A1 and UGT1A6 proteins influence the in vivo metabolism of APAP. Furthermore, to examine whether there is a gender-specific difference in the metabolism of APAP, we characterized the plasma concentration-time profiles of APAP and APAP glucuronide (AG) in male and female rats fed standard (SD) and HF1 diets.

There have been several studies on the regulation of UGT gene expression by transcriptional factors (Mackenzie et al., 2003). We have localized the PB response enhancer activity of human UGT1A1 to the 290-bp PB responsive enhancer module (gtPBREM) located at -3499/-3210, and have identified the nuclear receptor constitutive androstane receptor (CAR), pregnane X receptor (PXR), and glucocorticoid receptor (GR) as transcription factors that regulate gtPBREM (Sugatani et al., 2001, 2005). In addition, an aryl hydrocarbon receptor (AhR) response element and peroxisome proliferator-activated receptor α (PPARα) response element have also been reported to be localized within the 290-bp gtPBREM (Yueh et al. 2003; Senekeo-Effenberger et al., 2007). Previous studies on UGT1A6 gene expression documented that AhR and Nrf2 binding sites are located in the human UGT1A6 5'-regulatory region (-2052/-1), and hepatocyte nuclear factor 1α (HNF1α), CAR, and PXR binding sites were identified by computer-based homology analysis of the regulatory region (Krishnaswamy et al., 2005). Moreover, HNF4 α is a regulator of coordinated nuclear receptor (such as CAR and PXR)-mediated responses to xenobiotics

(Triona et al., 2003), and peroxisome proliferator-activated receptor-coactivator-1 α (PGC1 α) modulates the function of nuclear receptors such as CAR as a common coactivator (Puigserver and Spiegelman, 2003). To characterize gender-specific differences, we further examined the effect of the HF1 diet on the expression of transcription factors associated with sugar and lipid metabolism including PPARα and PPARγ that are as well as coactivator in liver cell nuclei.

## Materials and Methods

**Animals and experimental protocol.** All studies followed protocols approved by the Institutional Animal Care and Life Committee, University of Shizuoka. Male and female Wistar rats were obtained from Japan SLC (Hamamatsu, Japan) at 4 or 6 weeks of age and were acclimatized for 1 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 diet), high-fat and high-sucrose diet (HF1 diet), or high-fat diet (HF2 diet) for 2, 4 and 8 weeks. The HF1 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 (23.6% lipid, 57.4% carbohydrate and 18.9% protein [kJ]), the HF2 diet consisted of 23% casein, 19.8% corn oil, 19.8% lard, 6.7% mineral mixture, 1.4% vitamin mixture, 0.2% choline chloride, 5% cellulose and 24.1% sucrose (65.4% lipid, 17.7% carbohydrate and 16.9% 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% water (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.

Administration of acetaminophen: At 11 weeks of age after being fed the SD or HF1 diet for 4 weeks, animals were randomly assigned to treatment (APAP) or vehicle groups. APAP was dissolved in 37$^{o}$C warm 0.9% saline solution (100 mg/ml) with the pH adjusted to 10 with 0.1N NaOH and injected intraperitoneally at 500 mg/kg body weight between 8:00 AM and 9:00 AM. At different time points after APAP administration (0, 1, 2, 4, 8, and 24 h), blood was taken for measuring the plasma concentrations of APAP and AG.

Administration of 1,7-phenanthroline (PH): At 13 weeks of age after being fed the SD or HF1 diet for 8 weeks, male rats were randomly assigned to treatment (PH) (Sigma-Aldrich, St.

Louis, MO) or vehicle groups. PH was dispersed in 0.5% methylcellulose containing 0.1 M citric acid (75 mg/ml) and injected per os at 75 mg/kg body weight once daily for 3 days. The experiments were done 24 h after the last administration of PH.

Administration of phenobarbital: The SD-fed male rats at 11 weeks of age were administered phenobarbital (PB, Wako Pure Chemicals) dissolved in 0.9% sodium chloride (80 mg/kg) or vehicle by intraperitoneal injection once daily for 4 days between 8:00 AM and 9:00 AM. The experiments were done 4 h after the last administration of PB.

Administration of clofibrate: The SD-fed rats at 11 weeks of age were administrated clofibrate (CFB, Sigma-Aldrich) dissolved in corn oil (300 mg/ml/kg) or vehicle by intraperitoneal injection once daily for 5 days between 8:00 AM and 9:00 AM. The experiments were performed 4 h after the last administration of CFB.

**Blood and tissue sampling.** Rats were anesthetized with diethyl ether, and 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 immediately placed in liquid nitrogen. The median lobe of the liver was excised for RNA extraction and for the preparation of microsomes, plasma membranes and nuclear extracts. Aliquots were snap frozen and stored at –80$^{o}$C until analysis.

**Preparation of microsomes.** Liver microsomes were prepared by differential centrifugation, first at 900 g for 10 min, and then at 9,000 g for 15 min followed by 105,000 g 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.

**Membrane preparation.** Crude liver membranes were prepared as described previously (Ogawa et al., 2000). Liver (about 1.6 g) was homogenized in 5 volumes of 0.1 M Tris-HCl buffer (pH. 7.4) containing 1 μg/ml leupeptin and pepstatin A and 50 μg/ml PMSF with 20 strokes of a Dounce homogenizer. After centrifugation at 1,500 g for 10 min, the supernatant was centrifuged at 100,000 g for 30 min. The precipitate was suspended in 6 ml of the buffer and again centrifuged at 100,000 g for 30 min. The crude membrane fraction was resuspended in 0.1 M Tris-HCl buffer (pH 7.4) containing the above proteinase inhibitors using five strokes of a Potter homogenizer.

**Preparation of nuclear extracts.** Liver nuclear extracts were prepared as described previously (Sugatani, et al., 2006). 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 3 strokes of a Teflon-glass homogenizer . 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$^{o}$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 10 strokes of a Dounce homogenizer. Sodium chloride solution was added to the homogenate to bring the final concentration to 0.4 M and the homogenate was incubated at 4$^{o}$C for 30 min with gentle shaking. The supernatant after centrifugation at 105,000 g 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

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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 by electrophoresis on a sodium dodecyl sulfate-12.5% polyacrylamide gel and plasma membrane fractions (50 μg) were resolved on a sodium dodecyl sulfate-7.5% polyacrylamide gel, and electroblotted onto a polyvinylidene difluoride membrane (Millipore Corporation, Bedford, MA). The immunoblots were incubated with the following primary antibodies: rat CYP1A1: the antibody recognizes rat CYP1A1 and CYP1A2 (the molecular weight of rat CYP1A2 is lower than that of rat CYP1A1), rat CYP2B1, rat CYP2E1, rat CYP3A2, rat CYP4A1, and rat NADPH P450 reductase (CPR) (Daiichi Pure Chemicals Co., Tokyo, Japan), human MRP2 (sc-5770), human MRP3 (sc-5776), mouse CAR (sc-13065), mouse PXR (sc-7737), human RXRα (sc-551), human PPARα (sc-9000), human PPARγ (sc-7273), human AhR (sc-8088), human ARNT (sc-8076), human HNF1α (sc-6547), human HNF4α (sc-6556), human Nrf2 (sc-13032), human PGC1α (sc-13067), and human histone H1 (sc-8030) (Santa Cruz Biotechnology, Santa Cruz, CA), rat Oatp1 and rat Oatp2 (Alpha Diagnostic International, San Antonio, TX), chicken α-tubulin (Oncogen Research Products, Boston, MA), and rabbit antibodies against rat UGT1A1-, UGT1A6-, UGT1A7- and UGT2B1-specific peptides (Ikushiro et al., 1995, 1997). 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). The signal intensities were determined with a Fujix BAS-2000 bioimage analyzer (Fuji Photo Film).

**Determination of mRNA levels.** Total RNA was prepared from the liver or jejunum 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 a Prime Script RT reagent kit (Takara Bio. Inc., Ohtsu, Japan) 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., 2006) with an ABI PRISM 7000 Sequence Detector instrument (Applied Biosystems, Foster City, CA) using Premix Ex Taq reagent (TaKaRa Bio Inc.) for the TaqMan probe method or SYBR Premix Ex Taq reagent (TaKaRa Bio Inc.) for the intercalation reaction with SYBR Green I according to the manufacturer's specifications. The TaqMan probes and primers for rat *CAR* (NM_022941) (Hartley et al., 2004) and rat *UGT1A1* (NM_012683), *UGT1A6* (NM_057105) and *UGT2B1* (NM_173295) (Wang et al., 2003) were as reported previously, and those for rat *PPARα* (NM_013196) (assay identification number Rn00566193_ml) and rat *β-actin* (NM_031144) (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 *UGT1A7* (NM_130407) (Grams et al., 2000) and rat *β-actin* (Sugatani et al., 2006) were as reported previously. The thermal cycle conditions were as follows: incubate 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.** Serum glucose concentration was determined by the hexokinase

method using commercial reagents (R-Biopharm AG, Darmstadt, Germany). Serum insulin was measured using a double antibody radioimmunoassay kit specific for 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 and total cholesterol were measured enzymatically with kits from Shino Test (Tokyo, Japan). Frozen livers (about 0.5 g) were homogenized in 20 volumes (the SD group) or 100 volumes (the HF group) of 0.9% NaCl containing 0.1% Triton X-100, and the concentrations of triacylglycerol, total cholesterol, and nonesterified fatty acid were estimated with kits from Shino Test.

**Study of metabolism of APAP.** APAP and AG in plasma were determined using a modification of a method previously published (Esteban et al., 1992). To 50 μl of plasma, 5 μl of 5-hydroxy-L-tryptophan (2.5 μg) as an internal standard and 10 μl of 35% w/v perchloric acid were added. After centrifugation for 10 min at 2200 g, 10 μl of the supernatant was injected into an HPLC system, which consisted of a CO-8020 injector, DP-8020 pump, Mightysil RP-18GP Guard Pak precolumn cartridge, Mightysil RP-18GP (4.6 x 250 mm) reverse phase column, and a PD-8-20 spectrophotometer (TOSOH, Tokyo, Japan). Detection was performed by measuring absorption at 254 nm. An elution gradient was applied, beginning with 15% acetonitrile in an aqueous solution of 20 mM phosphate buffer (pH 4.5) at a flow rate of 1.0 ml. After 6 min, acetonitrile was increased to 30 % over 2 min. At 15 min, acetonitrile was increased to 70% over 2 min, and 5 min later was returned to 15%. Under these conditions, AG, internal standard, and APAP eluted at 2.8 min, 3.2 min, and 5.3 min, respectively. Standard curves for APAP and AG in plasma (2.5 – 800 μg/ml) were linear ($r \geq 0.997$).

**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**

*Nutritional status modulates hepatic expression of UGT mRNAs and proteins in male rats.* Consumption of the HF1 diet by male rats for 2 to 8 weeks and the HF2 diet for 8 weeks resulted in major increases in the absolute liver weight, relative liver weight, and liver lipids including a marked accumulation of triacylglycerols, total cholesterol and free fatty acids in the livers, with higher accumulation of lipids by the HF1 diet than the HF2 diet at 8 weeks (Fig. 1). Male rats fed the HF1 and HF2 diets for 8 weeks showed hepatic steatosis, which was not associated with an increase in serum AST, ALT and γ-GTP levels (data not shown). Table 1 shows that mRNA levels of UGT1A1, UGT1A6 and UGT1A7 were increased and the UGT2B1 mRNA level tended to increase in the liver but not the jejunum of male rats fed the HF1 diet for 8 weeks. The UGT1A1, UGT1A6, UGT1A7, and UGT2B1 mRNA levels did not increase in male rats fed the HF2 diet. To determine whether the observed changes in mRNA levels translated into altered hepatic protein levels, we determined the protein levels by Western blot analysis (Fig. 2). Consistent with the changes in the mRNA levels, the protein levels of UGT1A1, UGT1A6, UGT1A7 and UGT2B1 were significantly increased by consumption of the HF1 diet for 8 weeks (Fig. 2). A decrease in the protein level of CYP1A2, an increase in the UGT1A1 protein level and a tendency toward an increase in the UGT1A6 protein level in the liver were found after 2 weeks of consumption of the HF1 diet. In contrast, the change in the protein levels of CYP3As and UGT1A7 in the liver was dependent on the intake period of the HF1 diet; the levels of these proteins in the HF1 diet group were decreased at 2 weeks and increased at 8 weeks, whereas in the HF2 diet group they were decreased at 8 weeks (Fig. 2). The hepatic microsomal contents of CYP2E1 and

CYP4As, which is considered to function in the microsomal catalysis of lipid peroxides in nonalcoholic steatohepatitis (Leclercq et al., 2000), were significantly increased in the HF2 diet group but not in the HF1 diet group (Fig. 2).

*Gender-specific difference in UGT protein levels in livers of rats fed the SD or HF1 diet.* Consumption of the HF1 diet resulted in a major increase in liver lipids, including triacylglycerols, total cholesterol, and free fatty acids, after 4 weeks of the dietary regimen in both female and male rats, with the exception that serum glucose levels were significantly increased in male rats (1.53 $\pm$ 0.05 g/L in HF1-diet-fed male rats and 1.38 $\pm$ 0.03 g/L in SD-diet-fed male rats, $p<0.01$) but not in female rats (1.40 $\pm$ 0.02 and 1.43 $\pm$ 0.03 g/L in SD- and HF1-diet-fed rats, respectively). Liver baseline levels of CYP1A2, UGT1A7, and UGT2B1 mRNAs and proteins were higher in female rats than in male rats (Fig. 3 and supplemental Fig. 1). Intake of the HF1 diet for 4 weeks decreased the CYP1A2 mRNA and protein levels in livers of male and female rats, and increased UGT1A1 and UGT1A6 mRNA and protein levels in livers of male rats but not female rats (Fig. 3 and supplemental Fig. 1). UGT1A7 and UGT2B1 mRNA and protein levels in female rats were decreased to similar levels as those in control male rats by intake of the HF1 diet for 4 weeks. In contrast, there was no significant change in protein levels of CYP2E1, CYP3As, or CPR in male and female rats after consumption of the HF1 diet (Fig. 3).

*Effect of HF1 diet on the hepatic expression of drug transporters*. Changes in the protein levels of several basolateral and canalicular hepatic transporters were assessed in male and female rats after consumption of the HF1 diet for 4 weeks (Fig. 4). In rats fed the SD diet, protein expression significantly differed between genders and was higher (Mrp3), comparable (Mrp2) or lower (Oatp1, Oatp2) in female rats than in male rats (Fig. 4). No significant

changes of the Oatp1, Mrp2, or Mrp3 protein levels in male and female rats were detected after consumption of the HF1 diet, whereas the Oatp2 protein level tended to increase in the male rats and decrease in the female rats. Western blot immunostaining for α-tubulin confirmed equivalent protein loading.

*Plasma concentrations of acetaminophen (APAP) and its glucuronide (AG) after administration of APAP to male and female rats fed the SD or HF1 diet for 4 weeks.* APAP is mainly metabolized at therapeutic doses through glucuronidation and is also oxidized by P450s (rat CYP1A1, CYP1A2 and CYP2E1) to NAPQI, which reacts with cellular macromolecules and causes hepatic injury. To investigate whether the increases in UGT1A1 and UGT1A6 protein levels affected the in vivo metabolism of APAP in male and female rats fed the HF1 diet, we determined the plasma concentrations of APAP and AG. Figure 5 shows plasma concentration - time profiles of APAP and AG in male and female rats fed the SD and HF1 diets and then administered APAP at a dose of 500 mg/kg. The maximum plasma concentration of APAP was detected 1 h after APAP administration except in female rats fed the HF1 diet, in which APAP peaked at 2 h. The maximum plasma concentration of the metabolite AG was reached 2 h after APAP administration in the 4 groups. The maximum plasma concentration of APAP in male rats fed the HF1 diet was 1.4-fold higher than in male rats fed the SD diet. No significant difference in area under the plasma concentration – time curve (AUC) of APAP was found in male or female rats fed the SD and HF1 diets (Fig. 5). In contrast, consistent with the elevation in UGT1A1 and UGT1A6 but not CYP1A2 and CYP2E1 protein levels, the AUC of AG in male rats fed the HF1 diet was elevated 2.1-fold compared to male rats fed the SD diet, whereas there was no significant difference in the AUC of AG between female rats fed the SD and HF1 diets.

*Gender-related differences in baseline and diet-induced expression of transcription factors in the rat liver.* We next investigated the effect of the SD and HF1 diets on the protein levels of transcription factors and their coactivator in liver cell nuclei. Western blot immunostaining for histone H1 confirmed equivalent protein loading. Figure 6 shows that there was no significant difference in nuclear protein levels of CAR, PXR, RXR, AhR, ARNT, HNF1α, HNF4α, or PGC1 α in male and female rats fed the SD diet. Interestingly, the baseline nuclear protein levels of Nrf2, PPAR α, and PPARγ in male rats were significantly higher than those in female rats. The nuclear protein levels of CAR and PPAR α but not PXR, RXR, Nrf2, PPARγ, AhR, ARNT, HNF1α, HNF4α or PGC1α, were increased in male rats by intake of the HF1 diet for 4 weeks (Fig. 6). There was no significant difference in the nuclear protein levels of CAR, PXR, RXR, Nrf2, PPAR α, PPARγ, AhR, ARNT, HNF4 α, or PGC1 α between female rats fed the SD and HF1 diets except that the HNF1 α protein level was increased in female rats by consumption of the HF1 diet.

We further examined the mRNA levels of CAR and PPARα in livers of male and female rats fed the SD and HF1 diets. The baseline mRNA level of PPARα in female rats was half of the level in male rats and the mRNA level of CAR and PPARα in male rats fed the HF1 diet significantly increased to 1.4- or 1.6-fold over the control level in male rats fed the SD diet respectively, whereas there was no significant difference in the mRNA levels of CAR or PPARα in female rats (data not shown).

*Effect of treatment of male rats with AhR ligand, CAR activator or PPARα ligand on the expression of P450 and UGT proteins in the liver.* Rat *CYP1A1* and *UGT1A6* include the xenobiotic response element (XRE) sequences (Auyeung et al., 2003), but intake of the HF1 diet decreased the CYP1A1 protein level and increased the UGT1A6 protein level (Figs. 2, 3 and 7). In order to investigate whether the responses of CYP1A1 and UGT1A6 to AhR

ligand 1,7-phenanthroline (PH) were influenced by intake of the HF1 diet, we examined the effect of PH on the protein levels of CYP1A1, CYP1A2, UGT1A1, and UGT1A6 in livers of male rats fed the SD and HF1 diets. Treatment of the SD-fed male rats with PH increased the liver protein levels of CYP1A1, CYP1A2, and UGT1A6, but not UGT1A1. While the extent of induction of CYP1A1 by PH was higher than that of CYP1A2, UGT1A6 was more markedly increased among these enzymes in male rats fed the SD diet. Moreover, treatment of the HF1-fed male rats with PH significantly elevated not only the protein levels of CYP1A1 and CYP1A2 that were decreased by the HF1 diet, but also the UGT1A6 protein level, to 12.6-, 4.1- and 4.3-fold the vehicle-treated control levels, respectively (Fig. 7).

Next, since the protein levels of CAR and PPARα were elevated in liver cell nuclei of male rats fed the HF1 diet, we examined the effect of the CAR activator phenobarbital (PB) on the protein levels of UGT1A1, UGT1A6, UGT1A7, and UGT2B1 compared with CYP2Bs (Fig. 8A), and the effect of the PPARα ligand clofibrate (CFB) on the protein levels of CYP1A2, UGT1A1, and UGT1A6 compared with CYP4As (Fig. 8B). Treatment of SD-fed male rats with PB increased the liver protein levels of UGT1A1, UGT1A6, and UGT2B1 to 1.3-, 4.2- and 2.5-fold over the vehicle-treated control levels; these extents of induction were similar to that of CYP2Bs (3.0-fold) (Fig. 8A). Furthermore, to investigate whether an increase in the PPARα protein level in liver cell nuclei was associated with decreased protein levels of CYP1A1 and CYP1A2 and increased UGT1A1 and UGT1A6 protein levels in male rats fed the HF1 diet, we treated male rats with the PPARα ligand clofibrate. As shown in Figure 8B, administration of clofibrate to male rats reduced the protein level of CYP1A2 to 20% of the control level in the liver. In addition, the protein levels of UGT1A1 and UGT1A6 in the liver after treatment with clofibrate were increased to 1.8- and 1.9-fold over the vehicle-treated control levels, respectively; the extents of induction were similar to that of CYP4As, whose induction by clofibrate is mediated via PPARα (Zhou et al., 2002) (Fig. 8B).

**Discussion**

In a previous study, we demonstrated that feeding rats the HF1 diet for 8 weeks produced severe hepatic steatosis that was associated with hepatic injury caused by xenobiotics such as phenobarbital and dexamethasone via induction of CYP2B and CYP3A (Sugatani et al., 2006). Since there was no inflammation or fibrosis in the livers of rats fed the HF1 diet, this animal model is not an experimental model of nonalcoholic steatohepatitis, but is still useful for investigating the effects of lipid accumulation on the expression of drug-metabolizing enzymes and drug transporters in the liver (Table 1 and Figs. 1 to 3). Although it has been reported that CYP2E1 is up-regulated in nonalcoholic steatohepatitis and plays a key role in the development of liver injury by initiating lipid peroxidation (Leclereq et al., 2000), intake of the HF1 diet did not increase the protein levels of CYP2E1 and CYP4As (Sugatani et al., 2006; Figs. 2 and 3). In contrast, increases in CYP2E1 and CYP4A protein levels were found in HF2-diet-fed rats (Fig. 2). These results suggest that alterations in nutritional status caused by consumption of a high-fat and high-sucrose diet (HF1 diet) or a high-lipid diet (HF2 diet) affect the expression of CYP2E1 and CYP4As.

There have been many epidemiological studies on the inducibility of hepatic CYP1A enzymes by diet and its association with the metabolism of environmental and dietary carcinogens. Murray (2006) reported that dietary indolyl glucosinolates and flavonoids induce CYP1A expression either by direct ligand interaction with AhR or by augmenting the interaction of AhR with XREs in CYP1A1 and other target genes. Ciprofibrate, a PPARα ligand widely used in the treatment of hyperlipidemia, has been reported to decrease the ethoxyresorfin-*O*-deethylase activity of CYP1A1 in rats (Makowska et al., 1990). In addition, Shaban et al. (2004) demonstrated that when Wistar rats were treated with the AhR

ligand Sudan III and the PPARα ligand clofibrate for 3 days, AhR protein expression, CYP1A1/2 mRNA and protein expression, and the related metabolic activities of ethoxy-resorufin-*O*-deethylase and methoxy-resorufin-*O*-deethylase were down-regulated in a PPARα-dependent manner. Since the PPARα protein level in liver cell nuclei was elevated in male rats fed the HF1 diet, the suppression of CYP1A1 and CYP1A2 may be PPARα-dependent (Figs. 2, 3, 7 and 8B).

This study further demonstrated that intake of the HF1 diet was associated with increases in hepatic UGT1A1 and UGT1A6 mRNA and protein levels (Table 1 and Figs. 2, 3 and 7). UGTs conjugate endogenous lipophilic substrates such as bilirubin, thyroxine, estradiol and serotonin as well as xenobiotics such as acetaminophen, morphine, nonsteroidal anti-inflammatory drugs, flavonoids, and carcinogens with glucuronic acid (Radominska-Pandya et al., 1999; Tukey and Strassburg, 2000). Environmental compounds such as certain fresh fruits and vegetables in addition to xenobiotics such as theraputic drugs have been found to be UGT inducers; stimulation of estradiol glucuronidation in human liver microsomes by soy isoflavone daizein has been reported to be associated with a reduction in the incidence of hormonal cancer by soy food (Pfeiffer et al., 2005). In addition, marked differences in tissue- and gender-specific expression patterns of UGTs have been demonstrated to exist in mice, potentially influencing drug metabolism and pharmacokinetics (Buckley and Klaassen, 2007). Our study has demonstrated a gender-related change in the diet-induced expression of UGT1A1 and UGT1A6 in rats (Fig. 3) for which the mechanism remains to be clarified.

The glucuronidation of APAP in humans has been reported to be due mainly to UGT1A1, UGT1A6, UGT1A9, and UGT2B15 (Mutlib et al., 2000). Relatively high levels of UGT1A1 and UGT1A6 are expressed in rat liver. In rats, UGT1A9 is a pseudogene whose function is considered to be replaced by UGT1A7 (Emi et al., 1995). A recent study

demonstrated that UGT1A6 and UGT1A7 possess similar kinetic properties regarding APAP catalysis, and their relative contributions in vivo are predicted to depend on their levels of expression, which are determined by environmental exposure (Kessler et al., 2002). To investigate whether the increase in UGT1A1 and UGT1A6 protein levels by intake of the HF1 diet affects the in vivo metabolism of drugs in rats, in this study we focused on the in vivo metabolism of APAP, which is predominantly metabolized to glucuronide and sulfate. APAP glucuronide (AG), a monovalent organic anion formed in hepatocytes, is a substrate for Mrp2, which is responsible for the biliary excretion, and Mrp3, which exports products of hepatic metabolism back into the sinusoidal blood (Xiong et al., 2002). Approximately 50% of AG formed in hepatocytes is excreted into the bile and the rest is exported into the blood via the basolateral membrane (Xiong et al., 2002). In this study, in male rats fed the SD and HF1 diets, no significant difference in the AUC of APAP was found, although the maximum concentration of APAP in the HF1 diet group was higher than that in the SD diet group (Fig. 5). However, the AUC of AG in the HF1 diet group of male rats was 2.1-fold higher than that in the SD diet group. In contrast, there were no significant differences in the AUCs of APAP or AG between female rats fed the SD and HF1 diets, in accord with the lack of a significant increase in the expression of UGT1A1, UGT1A6, and UGT1A7. These observations indicate that the elevation in the AUC of AG may reflect the enhancement of the in vivo glucuronidation of APAP by increased UGT1A1 and UGT1A6 protein levels, since no significant change in the expression of Mrp2 and Mrp3 proteins was found in HF1-diet-fed male rats (Fig. 4) and the protein levels of CYP1A1, CYP1A2, and CYP2E1, which oxidize APAP to produce NAPQI, were also not increased (Figs. 2, 3 and 7).

Transcriptional up-regulation of drug-metabolizing phase 1 enzymes by xenobiotics occurs via CAR (CYP2B induction), PXR (CYP3A induction), AhR (CYP1A induction) and PPARα (CYP4As induction). The drug-metabolizing phase II enzymes NAD(P)H: quinone

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oxidoreductase and UDP-glucuronosyltransferase, are also induced by the transcriptional factor Nrf2 (Shelby and Klaassen, 2006). Administration of the CAR activator PB to normal male rats increased UGT1A1 and UGT1A6 protein levels to a similar extent as CYP2Bs (Fig. 8A). Moreover, administration of the PPARα ligand clofibrate to normal male rats increased UGT1A1 and UGT1A6 protein levels to a similar extent as CYP4As (Fig. 8B). Intake of the HF1 diet decreased the protein levels of CYP1A1 and CYP1A2 and increased the protein levels of UGT1A1 and UGT1A6 in male rats, even though the CYP1A1 and UGT1A6 promoters include XRE sequences (Figs. 2, 3 and 7). In contrast, PH induced CYP1A1 and UGT1A6 in male rats fed not only the SD diet but also HF1 diet (Fig. 7). The present study has demonstrated that gender-related changes in CAR and PPARα expression as well as in UGT1A1 and UGT1A6 expression occur in the liver cell nuclei of rats fed the HF1 diet (Figs. 3 and 6). These observations indicate that UGT1A1 and UGT1A6 may be induced via CAR and/or PPARα but not via AhR, although CAR and PPARα binding sites were identified in human UGT1A1 (Sugatani et al., 2001; Senekeo-Effenberger et al., 2007) and thought to occur in human UGT1A6 but not in rat UGT1A1 and UGT1A6. Consumption of a high-fat and high-sucrose diet caused increased in blood glucose and liver lipids (text and supplemental Table 1). Dhe-Paganon et al. (2002) proposed that fatty acids or related molecules might be endogenous ligands for HNF4α, and that HNF4α may function as a biosensor for fatty acids within the cell. Glucose itself has been demonstrated to induce the expression of HNF4α mRNA in primary-cultured rat hepatocytes (Oyadomari et al., 2000). Additionally, Ding et al. (2006) demonstrated that HNF4α directly regulates CAR gene expression through an HNF4-responsive element in its proximal promoter in mice. Thus, the enhanced expression of CAR may be mediated by HNF4α activation through elevated blood glucose and accumulated liver fatty acids. The reason for the gender - related response of PPARα in HF1-diet-fed rats is currently unclear (Fig. 6). However, it is possible

that PPARα induced by intake of the HF1 diet may contribute to the increases in the protein levels of UGT1A1 and UGT1A6 and the decrease in the protein level of CYP1A2 in male rats, as these enzymes were similarly altered in clofibrate-treated male rats (Fig. 8B). Clofibrate and the HF1 diet exerted differential effects on the induction of CYP4As, UGT1A1, and UGT1A6, suggesting that transcriptional cofactors and/or endogenous molecules other than PPARα may contribute to the transcriptional regulation of these genes. In conclusion, we speculate that the HF1 diet caused the elevation in the expression and activation of CAR and PPARα in liver cell nuclei, which may be also associated with the concurrent alteration of UGT1A1 and UGT1A6. We are now investigating the molecular mechanism of rat UGT1A1 and UGT1A6 induction via CAR and PPARα and cross-talk between CAR and PPARα .

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Footnote

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**Legends for scheme and figures**

Fig. 1 Effects of high-fat and high-sucrose (HF1) diet and high-fat (HF2) diet on body weight, liver weight, and liver lipids in male rats. Rats (5 weeks of age) were fed SD, HF1 or HF2 diet for 2, 4 and 8 weeks, and then sacrificed. Values are the means ± S.E. of 3-6 determinations. *$p$<0.05, **$p$<0.01, ***$p$<0.001 for HF1 male rats versus SD male rats or HF2 male rats versus SD male rats; #$p$<0.05, ###$p$<0.001 for 4 or 8 week SD male rats versus 2 week SD male rats.

Fig. 2 Effects of high-fat and high-sucrose (HF1) diet and high-fat (HF2) diet on expression of hepatic drug-metabolizing enzyme proteins in male rats. Rats (5 weeks of age) were fed SD, HF1 or HF2 diet for 2, 4 and 8 weeks and then sacrificed, and the microsomal proteins (20 μg/lane) were prepared and subjected to immunoblot analysis. Relative levels were expressed by taking the control values obtained from 2 week SD male rats as 100. Values are the means ± S.E. of 3-4 determinations. *$p$<0.05, **$p$<0.01, ***$p$<0.001 for HF1 male rats versus SD male rats or HF2 male rats versus SD male rats; ##$p$<0.01, ###$p$<0.001 for 4 or 8 week SD male rats versus 2 week SD male rats.

Fig. 3 Effects of high-fat and high-sucrose (HF1) diet on expression of hepatic drug-metabolizing enzyme proteins in male and female rats. Rats (7 weeks of age) were fed SD or HF1 diet for 4 weeks and then sacrificed, and the microsomal proteins (20 μg/lane) were prepared and subjected to immunoblot analysis. Relative levels were expressed by taking the control values obtained from SD male rats as 100. Values are the means ± S.E. of 5 determinations. *$p$<0.05, **$p$<0.01, ***$p$<0.001 for HF1 male rats versus SD male rats or HF1 female rats versus SD female rats; #$p$<0.05, ###$p$<0.001 for SD female rats versus SD male rats.

Fig. 4 Effects of high-fat and high-sucrose (HF1) diet on expression of hepatic drug

transporter proteins in male and female rats. Rats (7 weeks of age) were fed SD or HF1 diet for 4 weeks and then sacrificed, and the plasma membrane fraction (50 μg/lane) was prepared and subjected to immunoblot analysis. Relative levels were expressed by taking the control values obtained from SD male rats as 100. Values are the means ± S.E. of 4-5 determinations. *$p$<0.05 for HF1 male rats versus SD male rats or HF1 female rats versus SD female rats; ##$p$<0.01, ###$p$<0.001 for SD female rats versus SD male rats; ++$p$<0.01, +++$p$<0.001 for HF1 female rats versus HF1 male rats.

Fig. 5 Plasma levels of acetaminophen (A) and its glucuronide (B) in male and female rats fed SD or HF1 diet for 4 weeks and then administered acetaminophen (500 mg/kg). Rats (7 weeks of age) were fed the 2 diets for 4 weeks and then administered acetaminophen (500 mg/kg), and then sacrificed. Values are the means ± S.E. of 5 determinations. *$p$<0.05, **$p$<0.01 for HF1 male rats versus SD male rats or HF1 female rats versus SD female rats.

Fig. 6 Effects of high-fat and high-sucrose (HF1) diet on expression of transcription factor proteins in the liver cell nuclei of male and female rats fed SD or HF1 diet for 4 weeks. Rats (7 weeks of age) were fed the 2 diets for 4 weeks and then sacrificed, and the nuclear proteins (30 μg/lane) were prepared and subjected to immunoblot analysis. Relative levels were expressed by taking the control values obtained from SD male rats as 100. Values are the means ± S.E. of 4 determinations. *$p$<0.05, **$p$<0.01 for HF1 male rats versus SD male rats or HF1 female rats versus SD female rats; ##$p$<0.01, ###$p$<0.001 for SD female rats versus SD male rats.

Fig. 7 Effects of 1,7-phenanthroline (PH) treatment on expression of CYP1A1, CYP1A2, UGT1A1, and UGT1A6 proteins in male rats fed SD or HF1 diet for 8 weeks. Male rats (13 weeks of age) were administered PH (75 mg/kg, p.o.) or vehicle (C)

once daily for 3 days and then sacrificed, and the microsomal proteins (20 μg/lane) were prepared and subjected to immunoblot analysis. Relative levels were expressed by taking the control values obtained from SD male rats as 100. Values are the means ± 3 determinants. *$p$<0.05, ***$p$<0.001 for PH-administered animals versus vehicle-administered animals.

Fig. 8 Effects of phenobarbital (A) or clofibrate (B) treatment on expression of hepatic CYP2Bs, UGT1A1, UGT1A6, UGT1A7 and UGT2B1 proteins (A) or hepatic CYP1A2, CYP4As, UGT1A1 and UGT1A6 proteins (B) in male rats fed the SD diet. Male rats (11 weeks of age) were administered phenobarbital (PB, 80 mg/kg, i.p.) or vehicle (0.9% sodium chloride) once daily for 4 days (A), or clofibrate (CFB, 300 mg/kg, i.p.) or vehicle (corn oil) once daily for 5 days and then sacrificed, and the microsomal proteins (20 μg/lane) were prepared and subjected to immunoblot analysis. Relative levels are expressed by taking the control values obtained from SD animals as 100. Values are the means ± S.E. of 5 determinations. *$p$<0.05, **$p$<0.01, ***$p$<0.001 for PB- or CFB-administered animals versus vehicle-administered animals.

Supplemental Fig. 1

Effects of high-fat and high-sucrose (HF1) diet on expression of drug-metabolizing enzymes in male and female rats fed SD or HF1 diet for 4 weeks. Rats (7 weeks of age) were fed the SD or HF1 diet for 4 weeks and then sacrificed. Total RNA was isolated and analyzed via quantitative real-time polymerase chain reaction as described in Materials and Methods. Relative levels were expressed by taking the control values obtained from SD male animals as 1. Values are the means ± S.E. of 5 determinations in each group. *$p$<0.05, **$p$<0.01, ***$p$<0.001 for HF1 male rats versus SD male rats or HF1 female rats versus SD female rats; #$p$<0.05, ##$p$<0.01,

###$p<0.001$ for SD female rats versus SD male rats.

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Table 1. Effects of high-fat and high-sucrose (HF1) diet and high-fat (HF2) diet on expression of UGT mRNAs in the liver and jejunum of male rats.

| Gene | Liver (Fold induction) | | | Jejunum (Fold induction) | | |
|---|---|---|---|---|---|---|
| | SD | HF1 | HF2 | SD | HF1 | HF2 |
| UGT1A1 | 1.00 ± 0.04 | 1.61 ± 0.25* | 0.87 ± 0.16 | 1.00 ± 0.18 | 1.13 ± 0.13 | 0.89 ± 0.09 |
| UGT1A6 | 1.00 ± 0.58 | 7.28 ± 2.44*** | 1.38 ± 0.44 | 1.00 ± 0.19 | 0.43 ± 0.09 | 0.71 ± 0.08 |
| UGT1A7 | 1.00 ± 0.27 | 1.51 ± 0.10* | 0.95 ± 0.15 | 1.00 ± 0.27 | 0.34 ± 0.10** | 0.80 ± 0.10 |
| UGT2B1 | 1.00 ± 0.09 | 2.34 ± 0.67 | 1.08 ± 0.19 | 1.00 ± 0.44 | 1.51 ± 0.72 | 0.78 ± 0.14 |

Rats (5 weeks of age) were fed a high-fat and high-sucrose (HF1) diet, a high-fat (HF2) diet, or a standard (SD) diet for 8 weeks, and then sacrificed. Values are the means + S.E. of 3-4 determinations. *$p$<0.05, **$p$<0.01, ***$p$<0.001 for HF1 or HF2 male rats versus SD male rats.

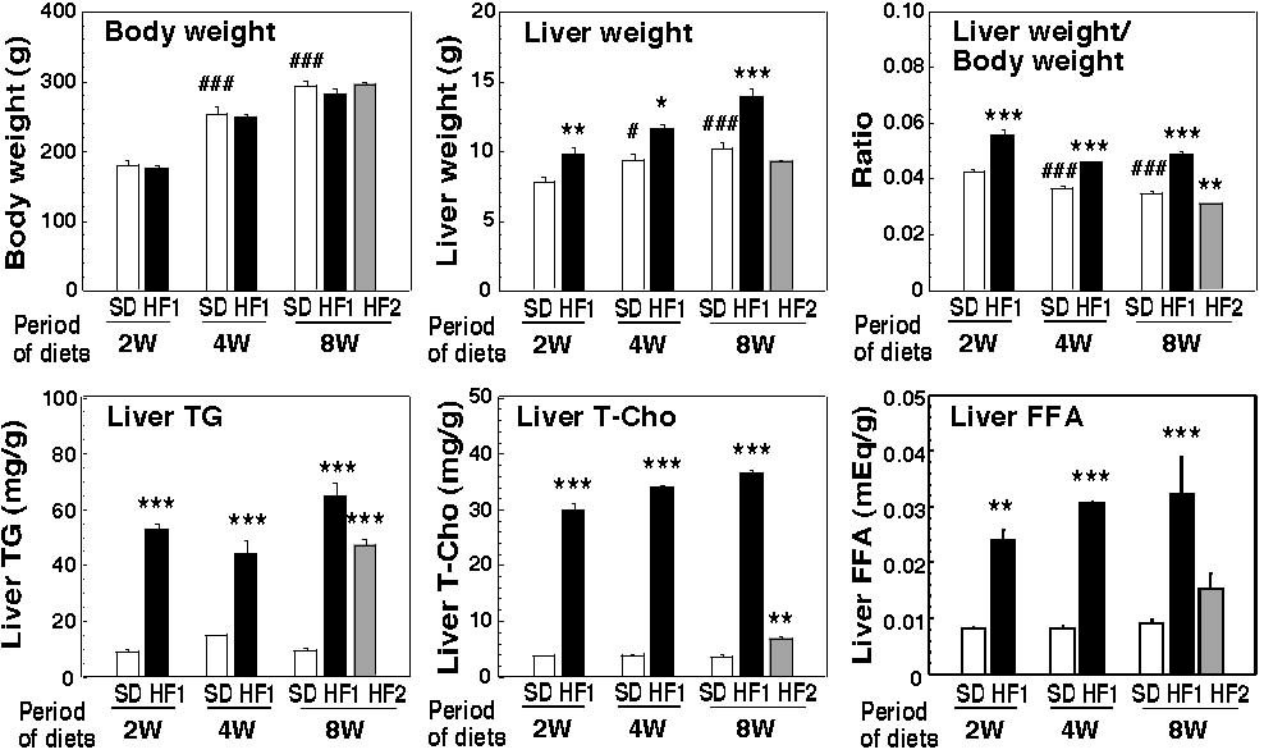
Body weight
Body weight (g)
400
300
200
100
0
###
###
SD HF1 SD HF1 SD HF1 HF2
Period of diets
2W 4W 8W
Liver weight
Liver weight (g)
20
15
10
5
0
**
#
*
###
***
SD HF1 SD HF1 SD HF1 HF2
Period of diets
2W 4W 8W
Liver weight/ Body weight
Ratio
0.10
0.08
0.06
0.04
0.02
0
***
###
***
###
***
**
SD HF1 SD HF1 SD HF1 HF2
Period of diets
2W 4W 8W
Liver TG
Liver TG (mg/g)
100
80
60
40
20
0
***
***
***
***
SD HF1 SD HF1 SD HF1 HF2
Period of diets
2W 4W 8W
Liver T-Cho
Liver T-Cho (mg/g)
50
40
30
20
10
0
***
***
***
**
SD HF1 SD HF1 SD HF1 HF2
Period of diets
2W 4W 8W
Liver FFA
Liver FFA (mEq/g)
0.05
0.04
0.03
0.02
0.01
0
**
***
***
SD HF1 SD HF1 SD HF1 HF2
Period of diets
2W 4W 8W

Fig. 1

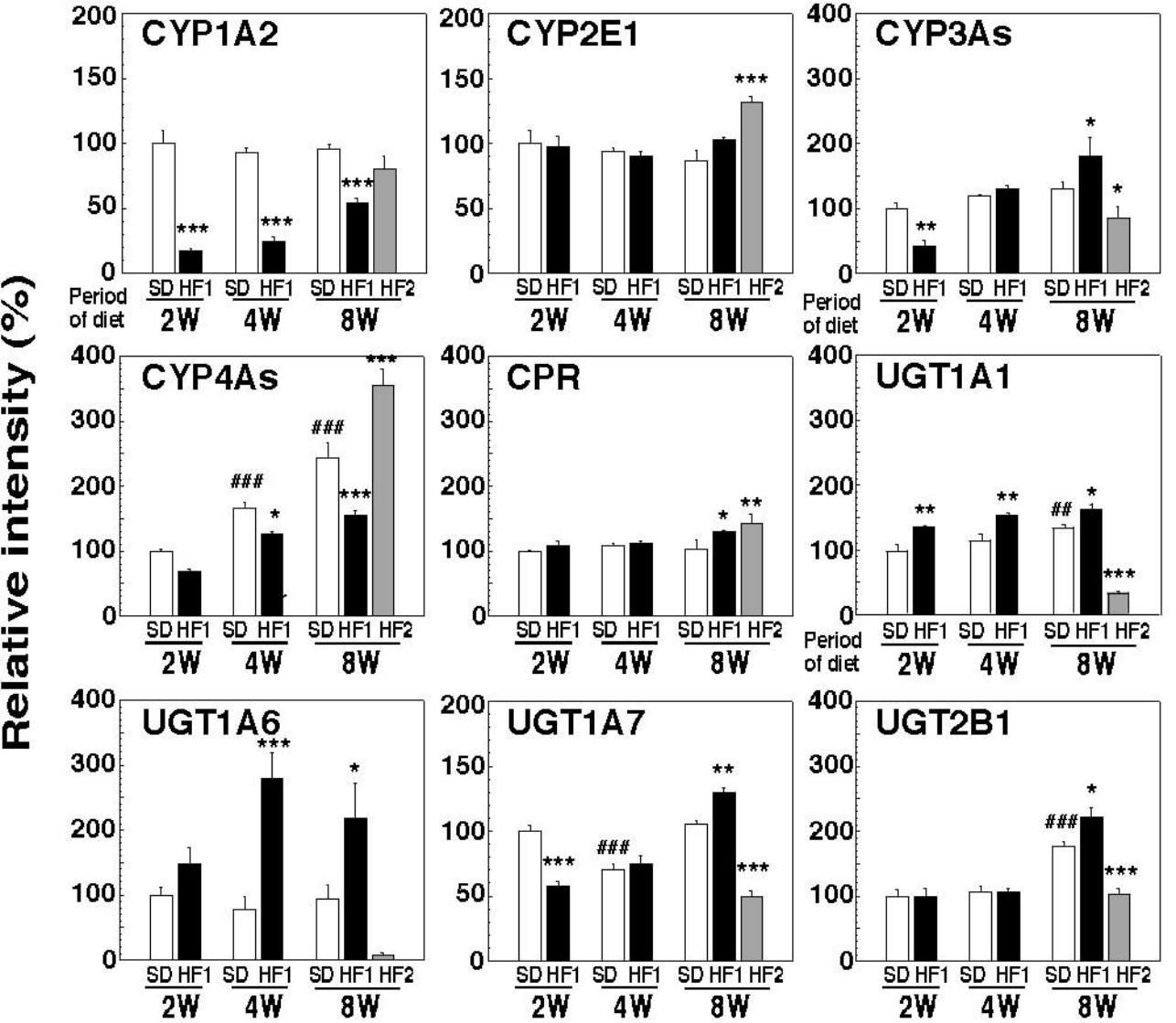
Relative intensity (%)
CYP1A2
CYP2E1
CYP3As
CYP4As
CPR
UGT1A1
UGT1A6
UGT1A7
UGT2B1
Period of diet
SD HF1 HF2
2W
4W
8W

Fig. 2

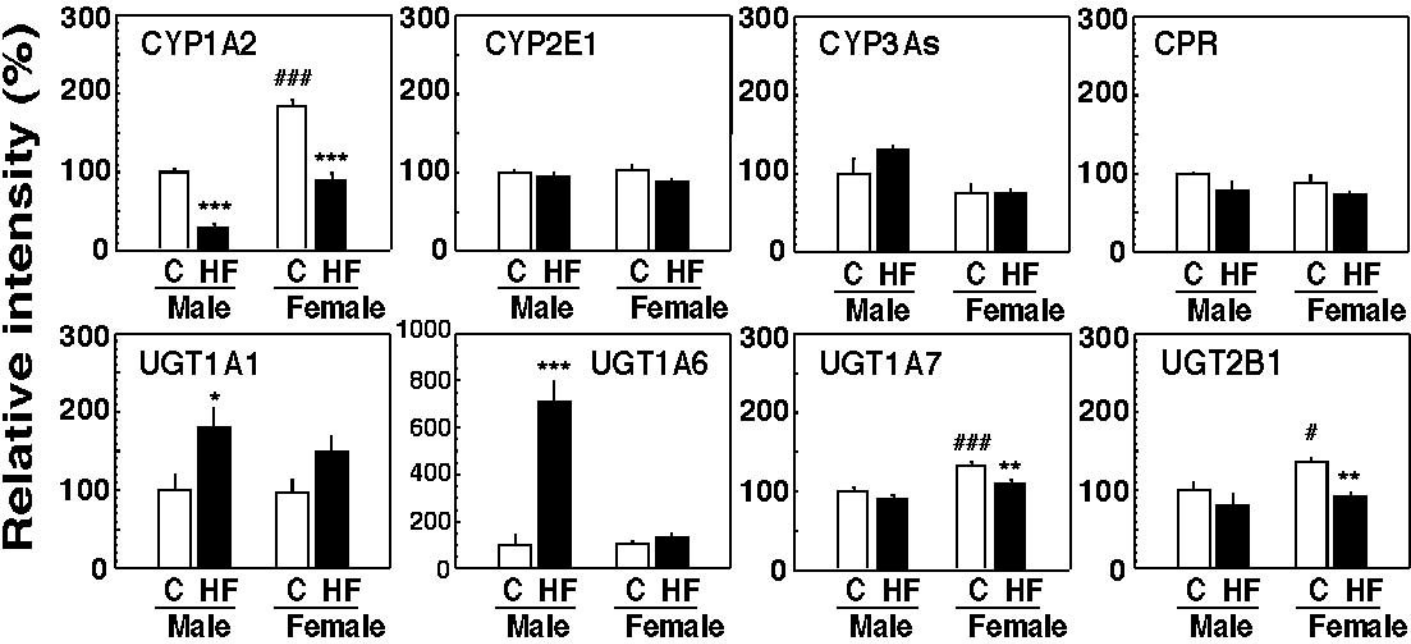
Relative intensity (%)
CYP1A2
CYP2E1
CYP3As
CPR
UGT1A1
UGT1A6
UGT1A7
UGT2B1
C HF
Male
Female
###
***
*
**
#

Fig. 3

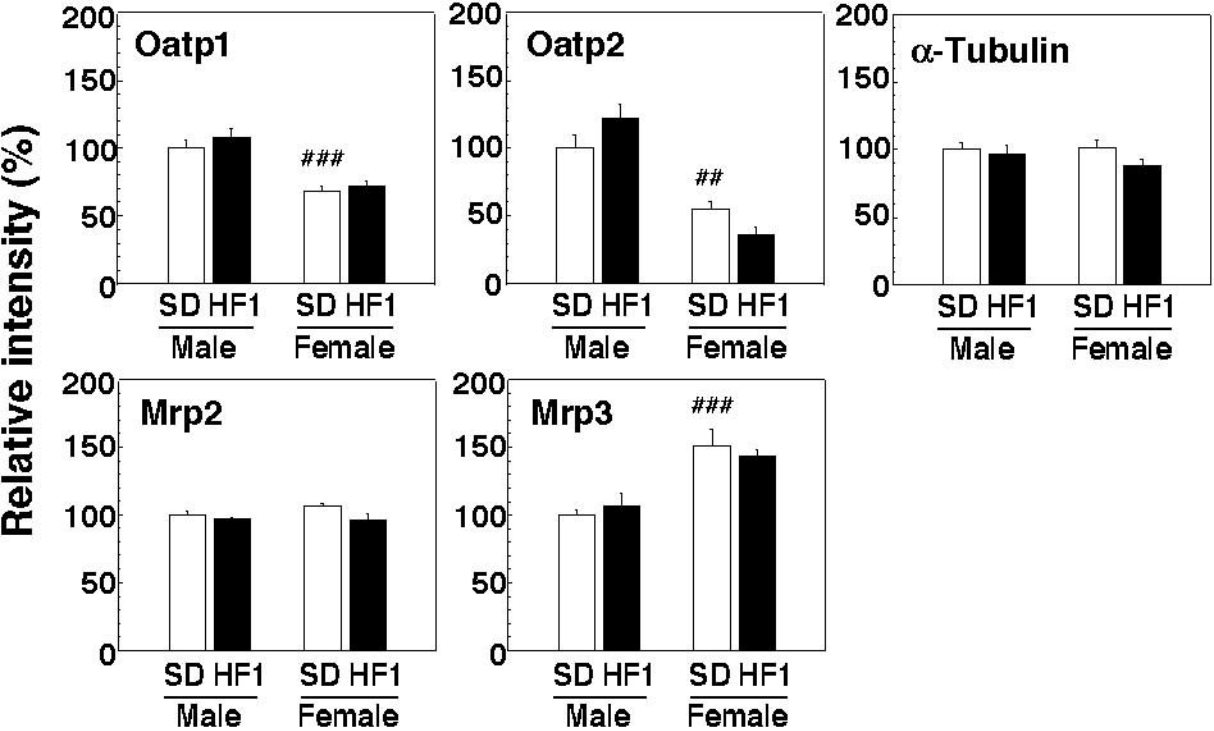
Relative intensity (%)
Oatp1
Oatp2
α-Tubulin
Mrp2
Mrp3
200
150
100
50
0
###
##
SD HF1
SD HF1
Male
Female

Fig. 4

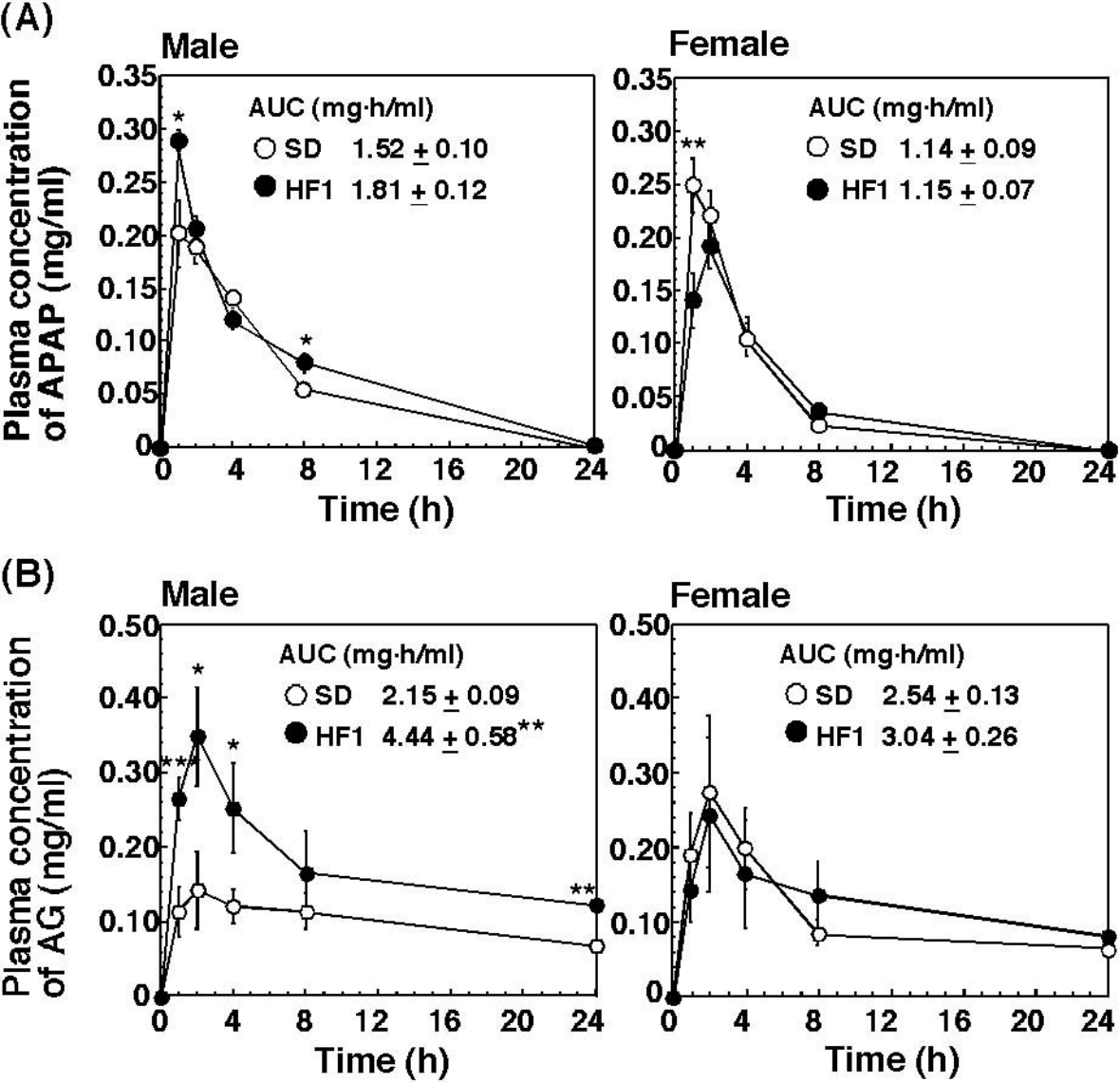
(A)
Male
Female
Plasma concentration of APAP (mg/ml)
AUC (mg·h/ml)
SD 1.52 ± 0.10
HF1 1.81 ± 0.12
SD 1.14 ± 0.09
HF1 1.15 ± 0.07
Time (h)
(B)
Plasma concentration of AG (mg/ml)
SD 2.15 ± 0.09
HF1 4.44 ± 0.58**
SD 2.54 ± 0.13
HF1 3.04 ± 0.26

Fig. 5

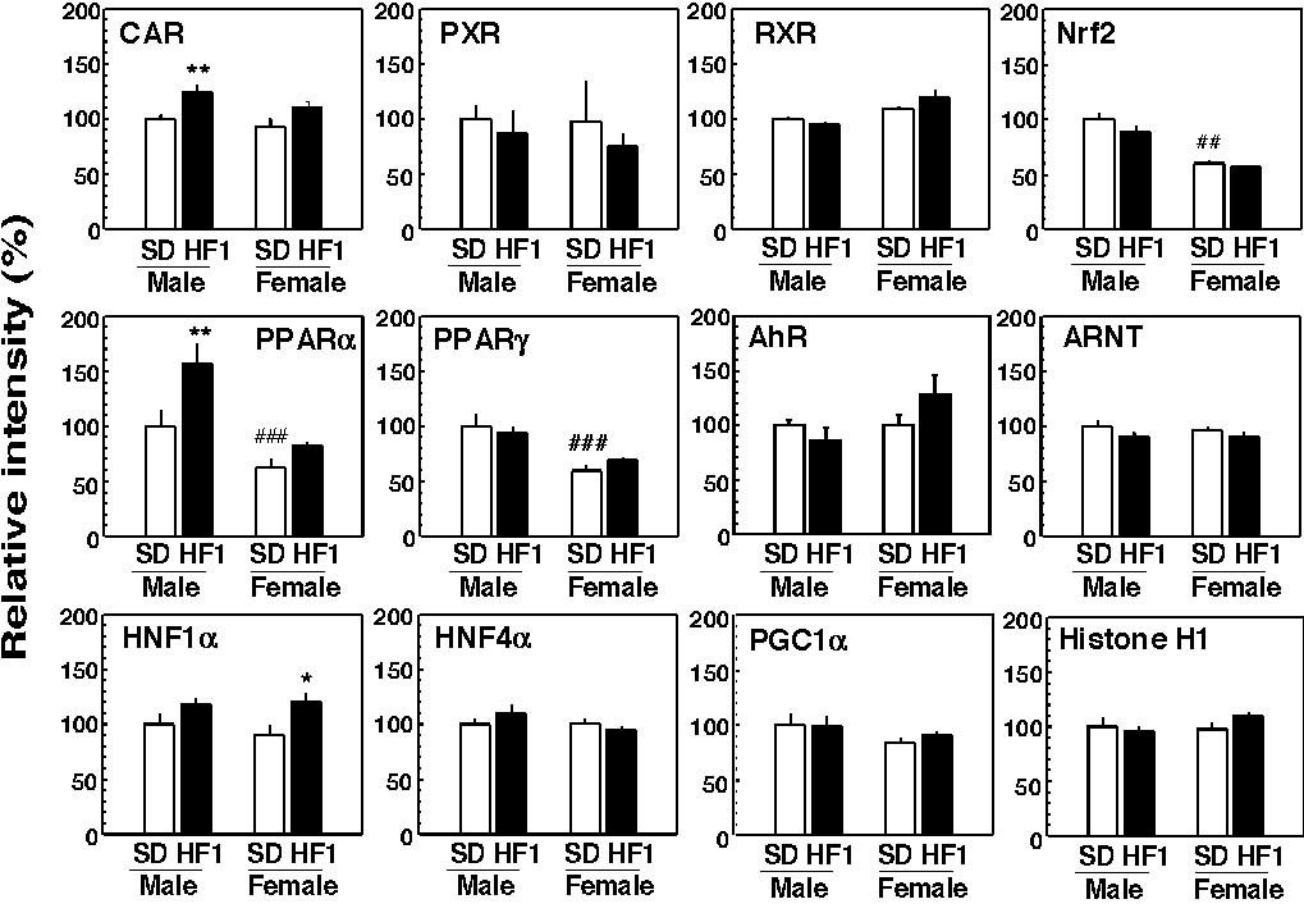
Relative intensity (%)
CAR
PXR
RXR
Nrf2
PPARα
PPARγ
AhR
ARNT
HNF1α
HNF4α
PGC1α
Histone H1
SD HF1
Male
Female
**
*
##
###

Fig. 6

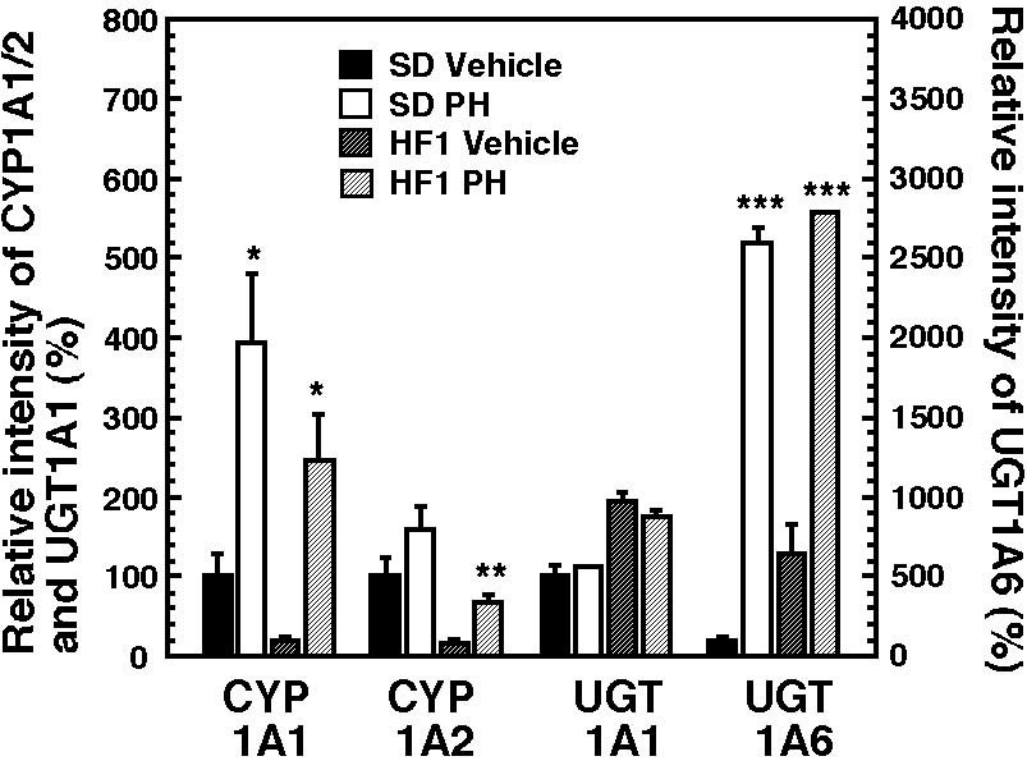
Relative intensity of CYP1A1/2 and UGT1A1 (%)
Relative intensity of UGT1A6 (%)
SD Vehicle
SD PH
HF1 Vehicle
HF1 PH
800
700
600
500
400
300
200
100
0
4000
3500
3000
2500
2000
1500
1000
500
0
*
*
**
***
***
CYP 1A1
CYP 1A2
UGT 1A1
UGT 1A6

Fig. 7

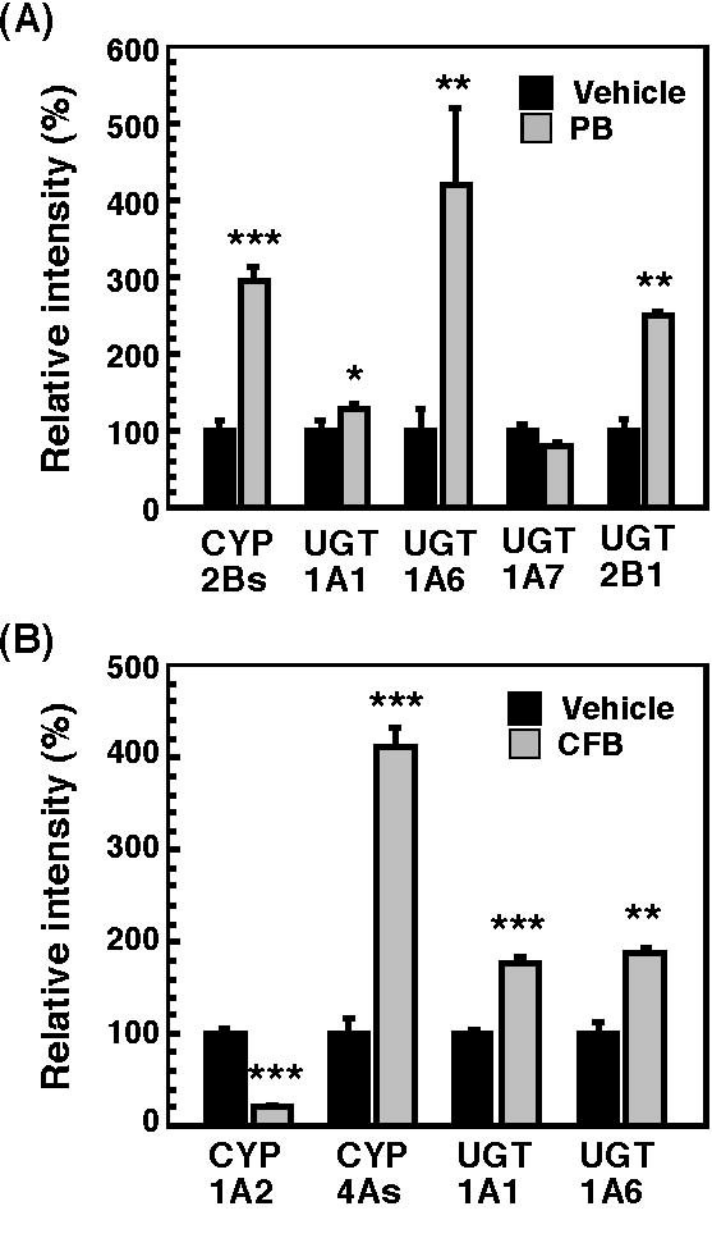
(A)
Relative intensity (%)
600
500
400
300
200
100
0
Vehicle
PB
***
*
**
**
CYP 2Bs
UGT 1A1
UGT 1A6
UGT 1A7
UGT 2B1
(B)
Relative intensity (%)
500
400
300
200
100
0
Vehicle
CFB
***
***
***
**
CYP 1A2
CYP 4As
UGT 1A1
UGT 1A6

Fig. 8