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Research Paper

Hepatic CYP3A Expression is Attenuated in Obese Mice Fed a High-Fat Diet

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Purpose. Changes in physiological, pathophysiological, and/or nutritional conditions often alter the expression of drug-metabolizing enzymes. In this study, we investigated obesity-induced changes in hepatic cytochrome P450 (P450) levels using nutritionally obese mice.
Methods. To induce obesity, mice were fed a high-fat diet or treated with gold thioglucose, which impairs ventromedial hypothalamus. Total RNAs and microsomal and nuclear proteins were prepared from the liver of these mice, and mRNA and protein levels of P450s and transcription factors were determined.
Results. Among P450s examined, the constitutive expression of CYP3As was drastically reduced at both mRNA and protein levels by nutrition-induced obesity. One-week administration of a high-fat diet also reduced hepatic CYP3As. However, changes in nuclear receptors involved in the transcriptional regulation of *CYP3A* genes were not correlated with that of CYP3As. Obese mice induced by gold thioglucose exhibited a different expression profile of hepatic P450s with no significant change in CYP3As.
Conclusion. High-fat diet-induced changes in energy metabolism, which eventually result in obesity, modulate the hepatic expression profile of P450s, particularly CYP3As. Alternatively, the accumulation of a certain component in a high-fat diet may directly attenuate the *CYP3A* expression, suggesting a clinically important drug–diet interaction.

KEY WORDS: CYP3A; cytochrome P450; high-fat diet; nuclear receptor; obesity.

INTRODUCTION

Activities of drug-metabolizing enzymes, such as cytochromes P450 (P450s), often show large interindividual differences for several reasons. Genetic polymorphism is one of the best-known reasons, and numerous gene variations affecting the expression level and/or catalytic activity of P450s and phase 2 enzymes have been reported, with *CYP2D6* and *CYP2C19* being studied the most (1). Mutant alleles such as *CYP2D6*5*, *CYP2C19*2*, and *CYP2C19*3* produce no active enzymes, making carriers poor metabolizers. Mutations in the promoter sequences of drug metabolism-related genes can also alter the expression level of those enzymes. We have recently identified a single nucleotide polymorphism in the promoter region of human UDP-glucuronosyltransferase UGT1A1 (*UGT1A1*60*) and demonstrated that it is associated with hyperbilirubinemia (2).

In addition to the genetic background, exposure to chemicals such as therapeutic drugs, nutrients and environmental contaminants, and the physiological and pathophysiological status modulate the expression of these drug-metabolizing enzymes. The mechanism for the chemical-induced gene expression of P450s and phase 2 enzymes has been demonstrated in detail, with nuclear receptor type transcription factors and a helix-loop-helix type transcription factor, aryl hydrocarbon receptor, playing pivotal roles. For example, phenobarbital (PB) and dexamethasone (DEX), prototypes of P450 inducers, activate constitutive androstane receptor (CAR) and pregnane X receptor (PXR), respectively, and the activated receptors form a heterodimer with retinoid X receptor (RXR) in the nucleus and bind to the PB-responsive enhancer module conserved in *CYP2B* genes or elements termed DR3, ER6, and xenobiotic-responsive enhancer module in *CYP3A* promoter sequences (3). On the other hand, it remains unknown how physiological/pathophysiolog-

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ABBREVIATIONS: CAR, constitutive androstane receptor; COUP-TF, chicken ovalbumin upstream promoter-transcription factor; DEX, dexamethasone; GST-hPXR-LBD, human PXR ligand-binding domain (amino acids 141–434) fused to glutathione *S*-transferase; GTG, gold thioglucose; HNF, hepatocyte nuclear factor; IL-6, interleukin-6; PB, phenobarbital; POR, NADPH cytochrome P450 reductase; PXR, pregnane X receptor; P450, cytochrome P450; RPS9, ribosomal protein S9; RT-PCR, reverse transcription-polymerase chain reaction; RXR, retinoid X receptor; STAT3, signal transducers and activators of transcription 3; TNF-α, tumor necrosis factor-α.

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ical conditions affect the activities of P450s and other enzymes (4).

Obesity is a worldwide concern as a risk factor for metabolic syndrome or syndrome X, including type 2 diabetes, cardiovascular disease, hypertension, and hyperlipidemia, and its increasing rate is epidemic. Therefore, it is of great interest to understand the effect of these conditions on the activities of drug-metabolizing enzymes because this may lead to serious drug–drug interactions and/or adverse effects in obese patients. Indeed, it is reported in genetically and drug-induced obese/diabetic model animals that the expression of phase 1 and 2 enzymes is altered in the liver, although the expression profile varies among model animals. For example, CYP2B and CYP3A levels in the liver of genetically obese Zucker rats were lower than those in control lean rats (5,6), whereas the levels of those P450s in obese *db/db* or *ob/ob* mice were comparable to or greater than those in control lean mice (7,8). Moreover, CYP2E1 is known as an enzyme that increases at both mRNA and protein levels in diabetes induced by streptozotocin or alloxan (9), and this enzyme also seems to be induced in obese and diabetic human livers (10,11); however, hepatic CYP2E1 levels are not altered in different diabetic model animals (12). It is therefore very important to choose an appropriate animal model to predict changes that could also occur in obese or diabetic humans.

Because most human obesity results from lifestyle-related matters and not genetic defects, we employed nutritionally obese animals fed a high-fat diet to investigate obesity-associated changes in the expression of P450s in the liver. Here, we demonstrate that CYP3A levels are reduced drastically in nutritionally obese mice and that this reduction seems to be associated with the administration of a high-fat diet because different expression profiles of hepatic P450s were observed between nutrition- and hypothalamic lesion-induced obese mice, and the short-term administration of a high-fat diet also caused CYP3A reduction.

METHODS

Materials

Erythromycin and DEX were purchased from Sigma (St. Louis, MO, USA). Monomethyl dimethylhydantoin, gold thioglucose (GTG), and corn oil were obtained from Wako Pure Chemical Industries (Osaka, Japan). Glucose 6-phosphate, $NADP^+$, and glucose 6-phosphate dehydrogenase were from Oriental Yeast (Tokyo, Japan). Antibodies against rat CYP1A2, CYP2B1, CYP2C6, rat CYP2E1, CYP4A1, and rat NADPH cytochrome P450 reductase (POR) were purchased from Daiichi Pure Chemicals (Tokyo, Japan). The anti-CYP3A4 antibody was a generous gift from Dr. Yasushi Yamazoe (Tohoku University, Sendai, Japan). Anti-hepatocyte nuclear factor-4α (HNF-4α), anti-chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII), and anti-RXRα antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-signal transducers and activators of transcription 3 (STAT3) and anti-p65 antibodies were from BD Biosciences (San Jose, CA, USA) and Stressgen (Victoria, BC, Canada), respectively. Streptavidin magnetic beads, Klenow fragment, and restriction enzymes were purchased from New England Biolabs (Beverly, MA, USA). The oligonucleotides used were synthesized by Kurabo (Osaka, Japan), and their sequences are shown in Table I.

Animal Treatment

Male ICR mice and Wistar rats were obtained from Japan SLC (Hamamatsu, Japan). All animals were used after acclimation for at least a week. They were given food (regular chow or high fat) and tap water *ad libitum* and maintained in a light–dark cycle of 12 h (light on at 8 a.m.). To induce obesity nutritionally, mice (5 weeks old) were given a high-fat diet from Oriental Yeast (referred to as HFD1 in this study; 36% fat, 22% protein, and 35% carbohydrate; the composition is shown in Table II) for 5 weeks or a high-fat diet HFD32 (referred to as HFD2 in this study; 32% fat, 26% protein, and 29% carbohydrate; the composition is shown in Table II) from Clea Japan (Tokyo, Japan) for 40 days. Control mice were fed the regular chow MF (Oriental Yeast; 5% fat, 24% protein, and 54% carbohydrate) for the same period. A portion of animals was treated with DEX (100 mg/kg) or vehicle (corn oil) intraperitoneally 24 h before killing. In short-term studies, 9-week-old mice or rats were fed HFD1, HFD2, or regular MF diet for 7 days. To induce obesity without a high-fat diet, mice (5 weeks old) were treated with GTG (300 mg/kg) or vehicle (saline) intraperitoneally, and were kept on regular chow for 4 weeks. Under ether anesthesia, blood was collected from the inferior vena cava in the fed state between 1 and 3 p.m. and then the animals were killed; the liver, small intestines, and epididymal white adipose tissue were excised for further analyses. All animal experiments were approved by the Laboratory Animal Committee of University of Shizuoka.

Measurement of Total P450 Content and Erythromycin *N*-Demethylase Activity

Hepatic microsomal fractions were prepared as described (13). For the preparation of intestinal microsomes, the jejunum was opened, the contents were removed, and then the tissue was rinsed in cold saline. Mucosa was collected by scraping with a glass slide and was homogenized in 3 volumes of 50 mM Tris–HCl (pH 7.5), 1.15% KCl, 1.7 mg/mL trypsin inhibitor (Type II-S, Sigma). Microsomal fractions were then prepared as for the liver. Total P450 contents in hepatic microsomal fractions were determined by the method of Omura and Sato (14). The incubation mixture (0.25 mL) of assays for erythromycin *N*-demethylation consisted of 0.1 M potassium phosphate buffer (pH 7.4), 0.1 mM EDTA, 250–400 μg of microsomal protein, and 2.5 mM erythromycin. The reaction was started by the addition of an NADPH-generating system (5 mM glucose 6-phosphate, 0.5 mM $NADP^+$, 1 unit of glucose 6-phosphate dehydrogenase, and 5 mM $MgCl_2$). The activity was calculated by the determination of formaldehyde formed by Nash reagent (15).

Table I. Sequences of Primers Used for RT-PCR

Gene	Sense primer	Antisense primer
CYP1A2[a]	ATGAGGAGCTGGACACGGTG	TCCACTGCTTCTCATCATGG
CYP2B1	TCACACCGGCTACCAACCCT	CTGTGGGTCATGGAGAGCTG
CYP2B2	TCTCACAGGCACCATCCCT	CTGTGGGTCATGGAGAGCTG
CYP2B10	AAAGTCCCGTGGCAACTTCC	CATCCCAAAGTCTCTCATGG
CYP2C6	ACAGCTAAAGTCCAGGAAGAG	CTGTCATGCAGCACTGATGAG
CYP2C11	ACAGCTAAAGTCCAGGAAGAG	TCATCATGCAGTATGGATGAC
CYP2C29	GCATGACAGCAAGGAGTTTC	TGTAGAAGGCATCACAGCAG
CYP2E1[a]	AGACCACCAGCACAACTC	AGGGCTGAGGTCGATATC
CYP3A1	GGAAATTCGATGTGGAGTGCC	AGGTTTGCCTTTCTCTTGCC
CYP3A2	ACTGCAGGAGGAGATCGATG	GTCAGGCTCCATTTATGACTG
CYP3A11	ACAAGCAGGGATGGACCTGG	TGTGACAGCAAGGAGAGGCG
CYP4A1	TCTGCACTGAGCTCCACC	GTGCTGGAACCACGGTTG
CYP4A2	TACAAGATCCCTGGATGG	CGATGCTGGAACCATTTC
CYP4A10	TATCACCTGGGATCACCTAG	CAGGAATGAGTGGCTGTGTC
β-Actin[a]	TCTACAATGAGCTGCGTGTGGC	AGCCAGGTCCAGACGCAGGAT
RPS9[a]	TCCGGAACAAACGTGAGG	TCCAGCTTCATCTTGCCC
Ugt1a1	CCTACGTGCCCAAGAGTTTG	ATCTCTGAGACCATGGATCC
Ugt2b5	CTCAATGCACTGGAGGAG	TGTCCAAGTGGTCTCAGG
St1a4	CCCAAAGTCTGGTACTACC	TGATCCAGCAGACTCTGAGG
St2a9	TCCAGGGTCACTCGGAAC	CTTTCATGGCTTGGAAGG
Mdr1a	TGGCCATCAGCCCTGTTC	AGAAAAGCTGCACCCATG
Mrp2	TGAGGATGAATCTCGACC	TGTCACTGTCCATGATGG
Mrp3	TGTGGGCTGTGGGAAGTC	CTCTCCAATCTCTGTCTG
Oatp4	TCTCTGAGAAGCATCCTTAC	TCCACTGGGTTCATTCCATC
HNF-4α	ATGAAGGAGCAGCTGCTGG	TCAGCCCCTTGGCATCTGG
COUP-TFII	CCTCAAAGTGGGCATGAG	GTCCGTGATCTGCAGGTC
PXR	ATTTCCGGCTGCCTGCAGTG	GTCCTCAATAGGCAGGTCCC
CAR	GGCATGAGGAAAGACATGATAC	CTTGAGAAGGGAGATCTGGTC
RXRα	CCCAGCTCACCAAATGAC	GATCAGCAGCTCGTTCCA
IL-6	GTTGCCTTCTTGGGACTGATG	CAATCAGAATTGCCATTGCAC
TNF-α	GCCTCTTCTCATTCCTGC	GTCTTTGAGATCCATGCC

All sequences are shown in 5′ to 3′ direction.
[a] Primers that can amplify both mouse and rat mRNAs.

Western Blotting for P450 Proteins

Microsomal proteins (liver, 5 μg; small intestine, 25 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel (9%) electrophoresis and transferred onto a polyvinylidene fluoride membrane (Immobilon-P; Millipore, Billerica, MA, USA). The membrane was incubated with primary antibodies and appropriate horseradish peroxidase-labeled secondary antibodies, and signals were detected with Lumi GLO reagent (Cell Signaling Technology, Beverly, MA, USA).

Reverse Transcription-Polymerase Chain Reaction

Total RNAs were prepared by the acid guanidine–phenol–chloroform method using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and first-strand cDNAs were synthesized with SuperScript III (Invitrogen) according to the manufacturers' protocols. Conventional and quantitative real-time polymerase chain reaction (PCR) experiments were carried out as described previously (13) using rTaq polymerase (Takara Bio, Ohtsu, Japan) and SYBR Premix Ex Taq (Takara Bio).

Table II. Composition of High-Fat Diets

Ingredient	Proportion (%)
HFD1	
Casein	24.0
L-Methionine	0.3
Lard	36.0
Cornstarch	10.0
Maltose	10.0
Sucrose	15.0
Vitamin mix, AIN-76A	1.0
Mineral mix, AIN-76	3.5
Choline bitartrate	0.2
HFD2	
Casein	24.5
Albumen	5.0
L-Cystin	0.4
Lard	15.9
Safflower oil	20.0
Cellulose	5.5
Maltodextrin	8.3
Sucrose	6.8
Lactose	6.9
Vitamin mix, AIN-93	1.4
Mineral mix, AIN-93G	5.0
Choline bitartrate	0.4

Western Blotting for Nuclear Proteins and DNA Affinity Purification

Total nuclear extracts were prepared from the pooled livers of control and obese mice as described previously (16). For the preparation of DNA affinity resin, *Cyp3a11* promoter DNA (from −279 to +6) was amplified by PCR using mouse genomic DNA as a template with primers 5′-cgggatccTTGAATGAAGCCAGCCTTGG-3′ and 5′-cgctcgagCTCTGCTGCACTGAGGACAG-3′ (lowercase nucleotides contain *Bam*HI and *Xho*I sequences for subcloning). The PCR product was subcloned into pCR2.1 plasmid (Invitrogen), and the sequence was confirmed by dideoxy sequencing (Macrogen, Seoul, Korea). After digestion of the plasmid with *Bam*HI and *Xho*I, the inserted promoter DNA was purified and biotinylated with biotin-14-dATP (Invitrogen) and Klenow fragment. The biotinylated DNA was then incubated with streptavidin beads. Affinity purification with the resin was performed as described (16). Total nuclear extracts (50 μg) and eluates corresponding to 250 μg of total extracts were subjected to Western blotting with 12% sodium dodecyl sulfate-polyacrylamide gel. Signals were detected as described above.

Preparation of Anti-CAR and Anti-PXR Antibodies

Anti-CAR antiserum was produced and purified as described previously (16). For anti-PXR antibody, human PXR ligand-binding domain (amino acids 141–434) fused to glutathione *S*-transferase (GST-hPXR-LBD) was expressed in BL21(DE3)pLysS cells (Promega, Madison, WI, USA) and was purified with glutathione Sepharose 4B resin (Amersham Biosciences, Piscataway, NJ, USA) from cleared cell lysates (soluble GST-hPXR-LBD) and inclusion bodies with the aid of sodium lauroyl sarcosinate (insoluble GST-hPXR-LBD), separately. Rabbits were immunized with either soluble or insoluble GST-hPXR-LBD, and antisera were collected from both rabbits and purified with Protein G Sepharose 4 Fast Flow (Amersham Biosciences), then with GST-hPXR-LBD immobilized onto glutathione Sepharose 4B beads. By Western blotting, both antisera reacted strongly with the immunogens. In addition, when mouse liver nuclear extracts were immunostained, similar patterns were observed with both antibodies, and the immunoreacted band indicated by arrowheads in Fig. 6B was also detected with anti-PXR.1 antibody (A-20; Santa Cruz Biotechnology) and bound to affinity resin with *Cyp3a11* promoter DNA (data not shown).

Other Methods

Protein concentrations were determined with Protein Assay (Bio-Rad, Hercules, CA, USA) with bovine serum albumin as the standard. Serum levels of glucose, total cholesterol, free fatty acid, and triacylglycerol were determined with kits for laboratory tests from Shino-Test (Tokyo, Japan) according to the manufacturer's instructions. To determine the hepatic lipid content, a piece of liver was homogenized in 20 volumes of phosphate-buffered saline containing 0.1% Triton X-100, and the homogenate was subjected to assays using the kits mentioned above. To analyze hepatic tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), a piece of liver was homogenized in 5 volumes of phosphate-buffered saline and centrifuged at 2500×*g* for 20 min, and the resulting supernatant was used for the immunoassay with kits from BioSource International (Camarillo, CA, USA). The significance of differences was assessed by Student's *t* test or one-way ANOVA followed by Scheffe's *post hoc* test using StatView software (Abacus Concepts, Berkeley, CA, USA).

Table III. Characteristics of Obesity Induced by a High-Fat Diet

	HFD1		HFD2	
Parameter	Control (n = 6)	Obese (n = 6)	Control (n = 6)	Obese (n = 8)
Body and tissue weight				
Body weight (g)	41.8 ± 3.2	49.9 ± 6.1*	43.6 ± 2.8	58.9 ± 5.1***
Liver weight (g)	2.0 ± 0.2	2.0 ± 0.3	2.3 ± 0.3	2.7 ± 0.4
Liver/body weight ratio (%)	4.8 ± 0.3	4.0 ± 0.5**	5.2 ± 0.4	4.6 ± 0.3**
WAT weight[a] (g)	0.7 ± 0.2	2.3 ± 0.7***	0.8 ± 0.2	3.4 ± 0.7***
WAT/body weight ratio (%)	1.6 ± 0.5	4.6 ± 1.0***	1.8 ± 0.6	5.7 ± 1.3***
Serum				
Glucose (mg/dl)	156 ± 31	213 ± 54*	209 ± 44	237 ± 76
Total cholesterol (mg/dl)	141 ± 18	196 ± 28**	120 ± 28	187 ± 53*
Triacylglycerol (mg/dl)	103 ± 29	136 ± 42	119 ± 41	121 ± 44
Free fatty acid (μEq/L)	778 ± 261	950 ± 195	589 ± 127	768 ± 197
Liver lipid				
Triacylglycerol (mg/g liver)	5.8 ± 1.4	23.0 ± 5.4***	14.1 ± 1.4	68.2 ± 20.4***
Total cholesterol (mg/g liver)	4.2 ± 0.3	4.4 ± 0.4	1.5 ± 0.4	2.2 ± 0.5*

Data are the means ± SD. Serum parameters and hepatic lipid contents were determined in duplicate for each mouse.
WAT = white adipose tissue.
*P < 0.05; **P < 0.01; ***P < 0.001 *vs.* corresponding control mice.
[a] Weight of epididymal white adipose tissue.

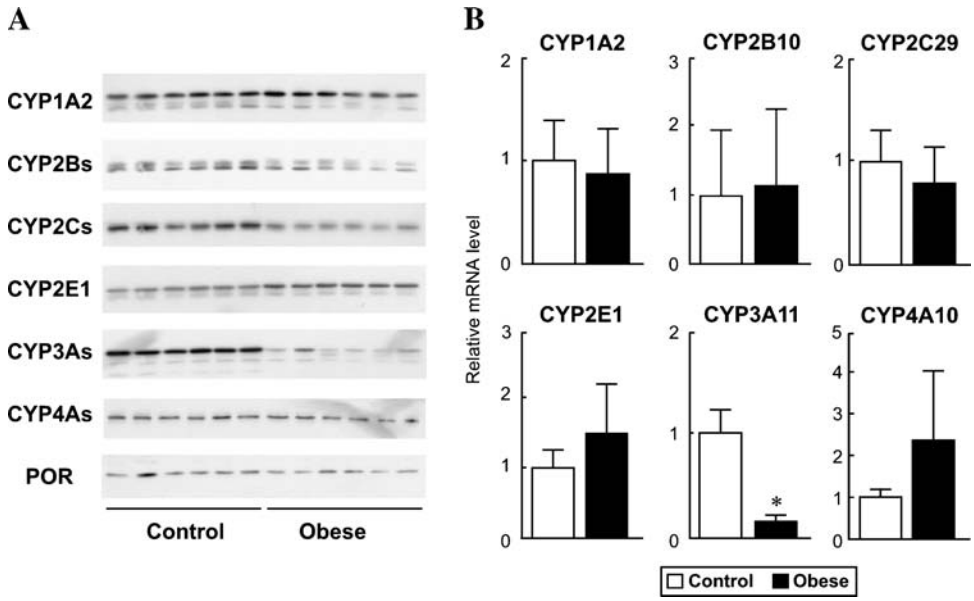

Fig. 1. Changes in the expression of hepatic cytochromes P450 (P450s) in nutritionally obese mice fed a high-fat diet HFD1. Mice were fed regular chow (Control; $n = 6$) or HFD1 (Obese; $n = 6$) for 5 weeks, and livers were dissected for further analyses. (A) Microsomal fractions were prepared individually and subjected to Western blotting (5 μg/lane) with antibodies against rat CYP1A2, CYP2B1, CYP2C6, rat CYP2E1, CYP3A4, CYP4A1, and rat NADPH cytochrome P450 reductase (POR). (B) Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analyses were performed with total RNA prepared from individual mouse livers. mRNA levels in each mouse were determined in duplicate, and the means ± SD ($n = 6$) are shown. P450 mRNA levels were normalized by that of β-actin, and mRNA levels of control mice were set at 1. *$P < 0.001$ *vs.* control mice.

RESULTS

Changes in the Hepatic P450s in Nutritionally Obese Mice

To examine the effects of nutrition-induced obesity on hepatic P450 levels, 5-week-old male ICR mice were fed a high-fat diet HFD1 (Table II) for 5 weeks. The average body weight of obese mice was 19% higher than that of mice fed a standard diet, and the mass of white adipose tissue also increased consistently (Table III). Serum glucose and total cholesterol concentrations were statistically higher in obese mice than in control mice, whereas no significant differences were

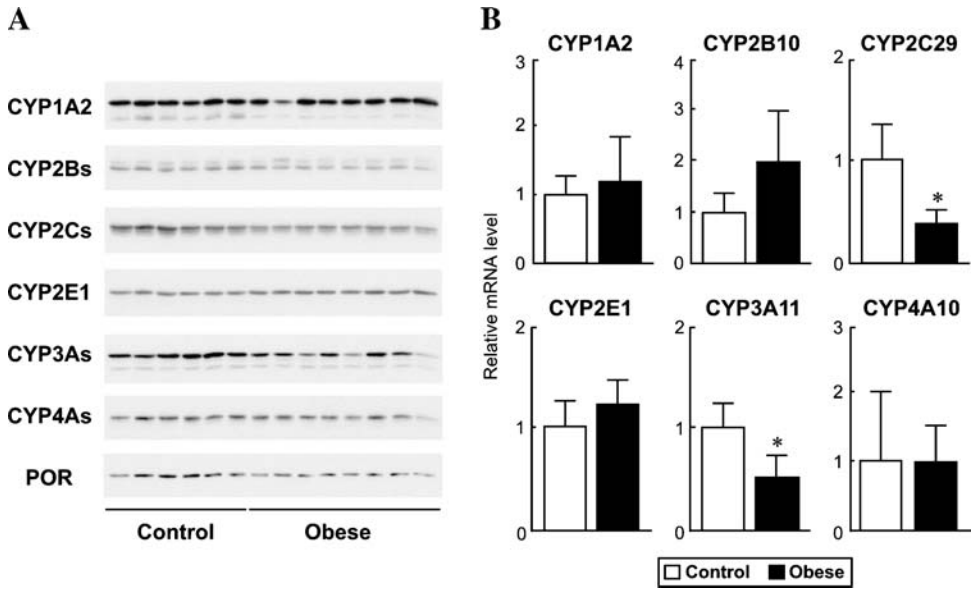

Fig. 2. Changes in the expression of hepatic P450s in nutritionally obese mice fed a high-fat diet HFD2. Mice were fed a regular chow (Control; $n = 6$) or HFD2 (Obese; $n = 8$) for 40 days, and livers were dissected for further analyses. Western blotting (A) and quantitative RT-PCR (B) were performed as described in the legend to Fig. 1 except that P450 mRNA levels were normalized by that of ribosomal protein S9 (RPS9). *$P < 0.01$ *vs.* control mice.

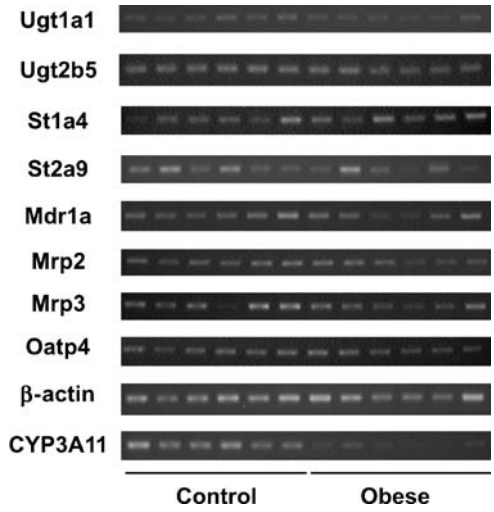

Fig. 3. Hepatic mRNA levels for phase 2 enzymes and drug transporters in nutritionally obese mice fed HFD1. RT-PCR analyses were performed with total RNAs prepared from livers of control mice or obese mice fed HFD1. Amplicons were electrophoresed on 1.2% agarose gels, stained with ethidium bromide, and detected under a UV light. The numbers of amplification cycles were 20 for β-actin and CYP3A11, 22 for Ugt1a1, Ugt2b5, and Oatp4, 24 for St1a4 and Mrp2, 28 for Mrp3, and 38 for St2a9 and Mdr1a.

observed in serum levels of triacylglycerol and free fatty acid (Table III). In the liver, triacylglycerol content was significantly greater in obese mice than in control mice (Table III).

Hepatic microsomal fractions were prepared from these mice, and total P450 contents were determined. The levels in control and obese mice were 0.45 ± 0.09 and 0.29 ± 0.04 nmol/mg protein (mean ± SD, $n = 6$ for both groups), respectively, and significant difference was observed ($P < 0.01$). Next, we performed Western blot analyses to identify a P450 form contributing to the reduction. Because antibodies against CYP2B1, CYP2C6, CYP3A4, and CYP4A1 used in this study could not distinguish mouse P450 forms in the corresponding subfamilies (i.e., CYP2B, CYP2C, CYP3A, and CYP4A, respectively), we hereafter describe the sum of immunoreactive proteins as CYP2Bs, CYP2Cs, CYP3As, and CYP4As, respectively. As shown in Fig. 1A, the most dramatic decrease was observed in CYP3As. Calculation of band intensities using NIH image software demonstrated that the average protein level in obese mice was about 18% that of control mice ($P < 0.0001$). In addition to CYP3As, the levels of CYP2Bs and CYP2Cs were also decreased by obesity, although the degree of reduction was much smaller compared with that of CYP3As. In contrast, the level of CYP2E1 protein was increased by obesity, consistent with human obesity (10,11). To confirm the reduction of CYP3As in obese animals, microsomal activities of erythromycin *N*-demethylation, which is catalyzed by CYP3As, were determined. As expected, the activity of obese mice (0.32 ± 0.20 nmol mg^{-1} protein min^{-1}, means ± SD, $n = 6$) was statistically lower ($P < 0.05$) than that of the control mice (1.24 ± 0.87 nmol mg^{-1} protein min^{-1}, means ± SD, $n = 6$).

To investigate whether the reduction of CYP3As occurred at mRNA levels, reverse transcription-PCR (RT-PCR) analyses were carried out. Although a number of P450 forms have been identified in mouse CYP2B, CYP2C, CYP3A, and CYP4A subfamilies, in this study, we amplified CYP2B10, CYP2C29, CYP3A11, and CYP4A10 mRNA as a representative of each subfamily. As shown in Fig. 1B, quantitative RT-PCR analysis demonstrated that CYP3A11 mRNA levels in obese animals were significantly lower than those of control mice ($P < 0.0001$). On the other hand, CYP2E1 and CYP4A10 mRNA levels tended to increase in obese animals, although there was no statistical significance.

To confirm the results obtained above, we tested another high-fat diet with a different composition of nutrients (HFD2, Table II). Male ICR mice (5 weeks old) were kept on HFD2 for 40 days to induce obesity. The administration of HFD2 caused more severe obesity compared with HFD1-fed mice in terms of their body weight, visceral adipose tissue mass, and accumulation of lipids in the liver (Table III).

Microsomal fractions were prepared from the livers of these mice, and total P450 contents were determined. The levels in control and obese mice were 0.48 ± 0.06 and 0.34 ± 0.03 nmol/mg protein (mean ± SD, $n = 6$–8), respectively, and significant difference was observed ($P < 0.001$). Western blotting and quantitative RT-PCR experiments demonstrated that both CYP3A proteins and CYP3A11 mRNA levels in the liver were markedly reduced in these obese mice as in HFD1-fed animals (Fig. 2), although the magnitude of their reduction was much greater in HFD1- than in HFD2-fed mice; calculation with NIH software demonstrated that the average CYP3As and CYP3A11 mRNA levels in obese mice

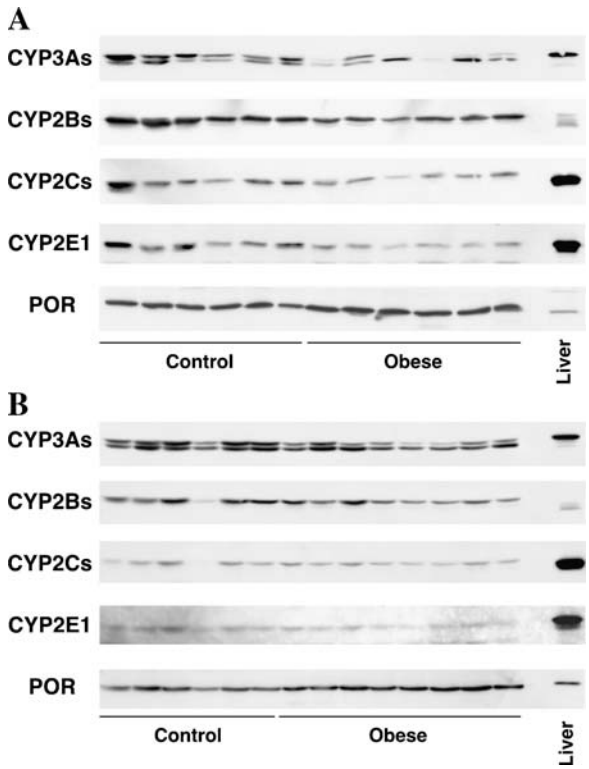

Fig. 4. P450 protein levels in the small intestine of nutritionally obese mice. Microsomal fractions were prepared individually from the small intestine of control and obese mice fed HFD1 (A) or HFD2 (B). A portion of the microsome (25 μg/lane) was subjected to Western blotting with antibodies against CYP3A4, CYP2B1, CYP2C6, rat CYP2E1, and rat POR. Hepatic microsomes (5 μg/lane) prepared from a control mouse were also loaded as a reference (lane Liver).

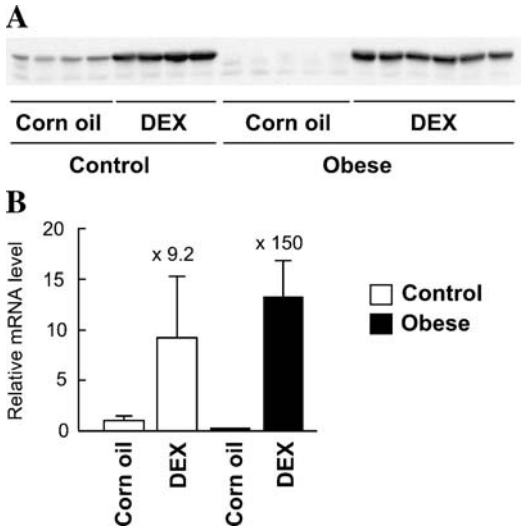

Fig. 5. CYP3A induction by dexamethasone (DEX) in the liver of nutritionally obese mice. Control and obese mice fed HFD1 were treated with DEX (100 mg/kg) or corn oil intraperitoneally 24 h before killing, and microsomes and total RNAs were prepared from the liver. (A) A portion of the microsome (5 μg/lane) was subjected to Western blotting with anti-CYP3A4 antibody. (B) Total RNAs were used for quantitative RT-PCR analyses. mRNA levels of CYP3A11 and β-actin were determined in duplicate for each mouse. CYP3A11 mRNA levels were normalized with β-actin levels, and those in corn oil-treated control mice were set at 1. Data represent the means ± SD (n = 4–6). Numbers above bars indicate the fold of induction by DEX.

were 53% ($P < 0.01$) and 51% ($P < 0.01$) that of control mice, respectively. In parallel, the microsomal activity of erythromycin *N*-demethylation in obese mice fed HFD2 (0.30 ± 0.10 nmol mg^{-1} protein min^{-1}, means ± SD, n = 8) was statistically lower ($P < 0.01$) than that in the control mice (0.86 ± 0.40 nmol mg^{-1} protein min^{-1}, means ± SD, n = 6). In addition to CYP3A11, CYP2C29 mRNA levels were also decreased in obese mice fed HFD2, consistent with the reduction of CYP2Cs (Fig. 2).

These results with two different high-fat diets indicate that nutritionally induced obesity affects the expression of hepatic P450s, especially CYP3A subfamily enzymes at both mRNA and protein levels.

mRNA Levels of Phase 2 Enzymes and Drug Transporters in Nutritionally Obese Mice

Because several phase 2 enzymes and drug transporters are regulated by mechanisms similar to those for *CYP3A* genes (3,17,18), we investigated whether obesity also decreased the expression of these genes. RT-PCR analyses with RNAs prepared from mice fed HFD1 demonstrated that hepatic mRNA levels of UDP-glucuronosyltransferases (Ugt1a1 and Ugt2b5), sulfotransferases (St1a4 and St2a9), and drug transporters (Mdr1a, Mrp2, Mrp3 and Oatp4/Slco1b2) were not affected significantly by obesity (Fig. 3), although there was a tendency toward decrease for Ugt1a1 and Oatp4 mRNAs. These results suggest that CYP3A subfamily enzymes seem to be the primary, if not only, target to change by nutrition-induced obesity among drug-metabolizing enzymes and drug transporters in the liver.

Changes in P450s in the Small Intestine of Nutritionally Obese Mice

In addition to the liver, the small intestine also expresses CYP3As and PXR abundantly (19,20) and plays an important role in the metabolism of exogenous compounds. We

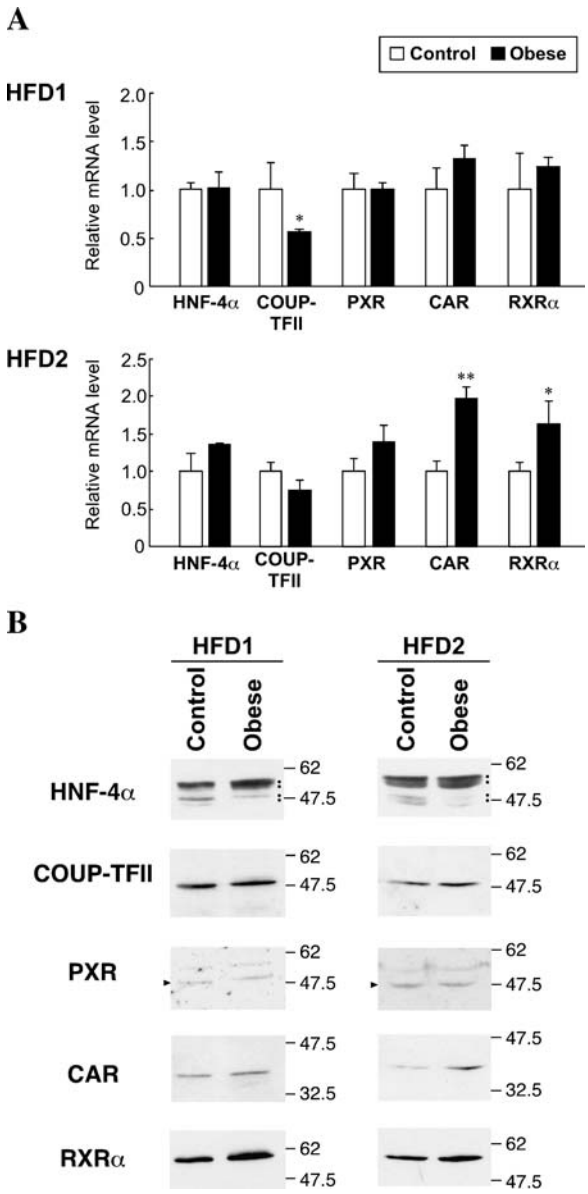

Fig. 6. Changes in the expression of nuclear receptors in the liver of nutritionally obese mice. (A) Hepatic mRNA levels of nuclear receptors were determined by quantitative RT-PCR in triplicate using pooled RNAs (n = 6–8). mRNA levels of nuclear receptors were normalized by that of RPS9. Relative mRNA levels were calculated by raising 2 to the power of differences in the mean threshold cycle numbers. Data represent the means ± SD of triplicate determinations with mRNA levels in control mice being set at 1. $^{*}P < 0.05$; $^{**}P < 0.01$ *vs.* corresponding control mice. (B) Nuclear extracts were prepared from pooled livers (n = 6–8) and were subjected to Western blotting (50 μg/lane) with the antibodies indicated. Molecular weights (in kilodaltons) are shown on the right. Bands indicated by arrowheads were also immunoreacted with anti-PXR.1 antibody (A-20; Santa Cruz Biotechnology) and were bound to *Cyp3a11* affinity resin (data not shown).

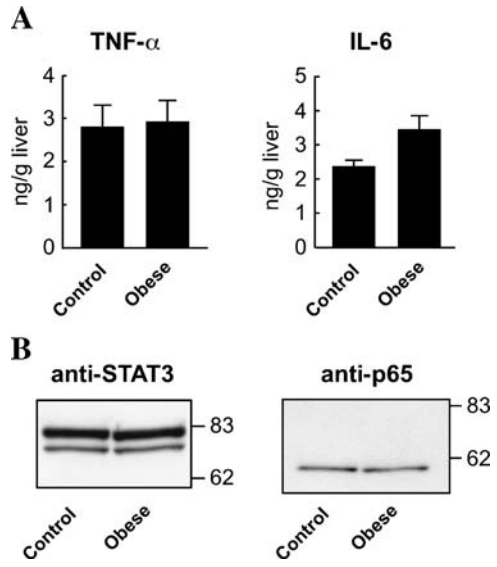

Fig. 7. Inflammatory signals in obese-mouse livers. (A) Livers were homogenized in phosphate-buffered saline and centrifuged briefly. Using the resultant supernatants, hepatic TNF-α and IL-6 protein levels in control and HFD1-fed obese mice were determined by ELISA. Each sample was measured in duplicate, and data are the means ± SD ($n = 6$). (B) The total nuclear extracts used in Fig. 6 were subjected to Western blotting with the antibodies indicated. Molecular weights (in kilodaltons) are shown on the right.

therefore investigated whether intestinal CYP3As were also down-regulated in these obese animals using microsomal fractions prepared from mouse small intestines. As shown in Fig. 4, Western blotting using anti-CYP3A4 antibody detected two immunoreactive bands. Whereas the upper bands corresponding to the major hepatic CYP3A form (probably CYP3A11) drastically decreased in both HFD1- and HFD2-induced obese mice, the lower bands were unaltered or declined moderately. Other P450s including CYP2Bs, CYP2Cs, and CYP2E1 were slightly reduced in obese animals. The amount of POR in the intestine was unchanged by obesity.

Effect of Obesity on CYP3A Induction by DEX

We have described the diminished expression of constitutive CYP3As in the liver of nutritionally obese mice. Because CYP3A enzymes are induced by a number of chemicals, we next investigated whether CYP3A induction was also impaired in these obese mice. Control and HFD1-fed obese mice were treated with DEX, a prototype of CYP3A inducers and a PXR activator, and then CYP3As and CYP3A11 mRNA levels were determined (Fig. 5). In control mice, DEX greatly increased the amount of hepatic CYP3As. Similarly, chemical treatment dramatically elevated CYP3As in obese mice to a level comparable to the control mice. The level of CYP3As in corn oil-treated obese mice was much lower than that in corn oil-treated control mice as shown in Fig. 1A. As expected from Western blotting results, quantitative RT-PCR demonstrated that CYP3A11 mRNA levels in DEX-treated obese mice were comparable to those in control mice treated with the inducer, although the fold of induction by DEX treatment was significantly greater in obese mice than in the control mice because of differences in constitutive levels. DEX treatment also increased CYP2Bs and CYP2B10 mRNA levels in both groups of mice to the same extent (data not shown).

Changes in Transcription Factors in the Liver of Nutritionally Obese Mice

Liver-enriched nuclear receptors including PXR, HNF-4α, CAR, and COUP-TF have been reported to be involved in the transcription of rat and human *CYP3A* genes (20–26). Recently, we reported that obese Zucker rats expressed CAR, a key regulator for CYP2B induction, at a significantly lower level than control lean rats, causing a defect in PB induction of CYP2Bs (6). We therefore investigated the possible role of nuclear receptors in the reduction of *CYP3A* expression in obese mice. Quantitative RT-PCR demonstrated that HNF-4α and PXR mRNA levels were not changed with obesity induced by either HFD1 or HFD2 diet (Fig. 6A). For other nuclear receptors, different changes were observed between HFD1- and HFD2-fed obese mice; COUP-TFII mRNA was reduced by 44% in HFD1-fed obese mice, and CAR and RXRα mRNAs were increased 2- and 1.6-fold, respectively, in HFD2-fed obese mice (Fig. 6A).

Next, total nuclear extracts were prepared from the livers, and nuclear protein levels of these transcription factors were determined (Fig. 6). As expected from RT-PCR results, CAR and RXRα proteins were increased in obese mice fed HFD2. In contrast, no significant difference was observed in COUP-TFII between control and HFD1-fed obese mice. RXRα was also increased in obese mice fed HFD1. With the anti-HNF-4α antibody, which reacts with the C terminus of HNF-4α, four bands (indicated by dots in Fig. 6B) were detected. Among them, the two minor bands with faster migration were decreased in both obese mice, whereas the two upper bands were increased in obese mice fed HFD1 but not in HFD2-fed mice. To investigate the DNA-binding ability of these transcription factors, nuclear extracts were

Table IV. Characteristics of Obesity Induced by Gold Thioglucose

Parameter	Control mice ($n = 5$)	Obese ($n = 4$)
Body and tissue weight		
Body weight (g)	41.3 ± 1.3	55.4 ± 9.9*
Liver weight (g)	2.0 ± 0.2	2.8 ± 0.9
Liver/body weight ratio (%)	4.7 ± 0.4	4.9 ± 0.7
WAT weight[a] (g)	0.7 ± 0.1	2.5 ± 0.8**
WAT/body weight ratio (%)	1.6 ± 0.3	4.4 ± 0.7***
Serum		
Glucose (mg/dl)	221 ± 28	246 ± 17
Total cholesterol (mg/dl)	151 ± 35	180 ± 40
Triacylglycerol (mg/dl)	131 ± 51	180 ± 64
Free fatty acid (μEq/L)	1074 ± 369	962 ± 99
Liver lipid		
Triacylglycerol (mg/g liver)	9.2 ± 2.0	27.7 ± 13.9*
Total cholesterol (mg/g liver)	2.0 ± 0.2	2.4 ± 0.2

Data are the means ± SD. Serum parameters and hepatic lipid contents were determined in duplicate for each mouse.
WAT = white adipose tissue.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs.* control mice.
[a] Weight of epididymal white adipose tissue.

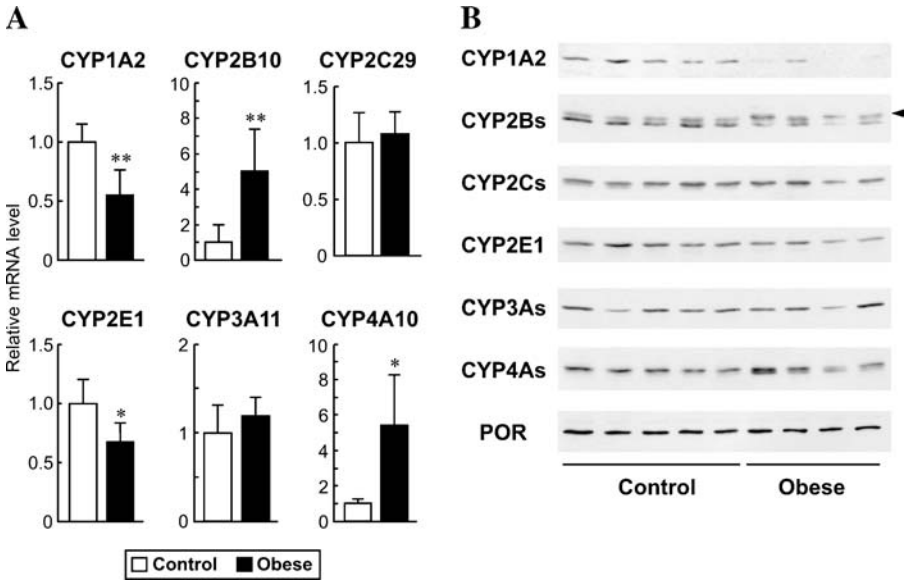

Fig. 8. Changes in the expression of hepatic P450s in gold thioglucose (GTG)-induced obese mice. Mice were treated with GTG or saline (vehicle) and kept on regular chow for 4 weeks. P450 mRNA (A) and protein (B) levels were determined by quantitative RT-PCR and Western blotting, respectively. An arrowhead indicates the band corresponding to CYP2B10. P450 mRNA levels were normalized with that of RPS9, and those of control mice were set at 1. Data represent the means ± SD (n = 4 or 5). *$P < 0.05$; **$P < 0.01$ *vs.* corresponding control mice.

incubated with affinity resin coupled with *Cyp3a11* promoter DNA (from −279 to +6), and bound proteins were probed with the antibodies used above. As a result, the same pictures as in Fig. 6B were obtained (data not shown). All HNF-4α bands were bound to the affinity resin (data not shown), although we do not know at present which form of HNF-4α protein contributes most to the expression of *CYP3A* genes. These results demonstrate that there was no notable change common to both obese mice fed either HFD1 or HFD2 to explain the decrease in the constitutive expression of

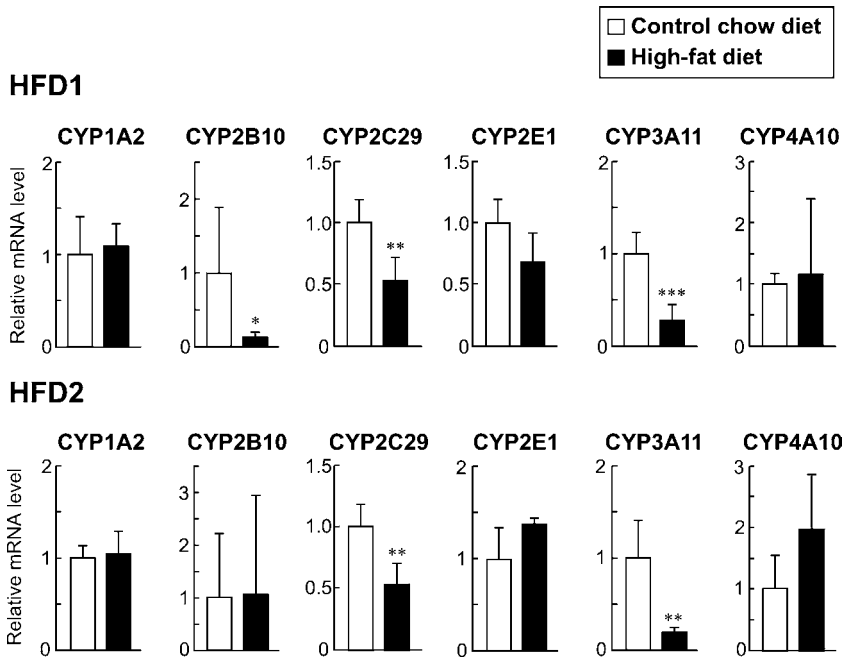

Fig. 9. Effects of 1-week administration of a high-fat diet on hepatic P450 mRNA levels in mice. Mice were fed regular chow or a high-fat diet, HFD1 or HFD2, for 1 week and hepatic P450 mRNA levels were determined by quantitative RT-PCR. mRNA levels were determined in duplicate for each mouse. P450 mRNA levels were normalized with that of RPS9, and those of control mice were set at 1. Data represent the means ± SD (n = 4–6). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ *vs.* corresponding control mice.

CYP3As, suggesting that these nuclear receptors play a minor, if any, role in the reduced expression of CYP3As associated with nutrition-induced obesity.

Roles of Proinflammatory Cytokines in CYP3A Down-Regulation

Obesity is associated with increased levels of TNF-α and IL-6 (27,28), and these proinflammatory cytokines have been reported to reduce the expression of P450s in the liver and in primary hepatocytes (29–31). We therefore examined the possibility that the down-regulation of P450s in obese animals observed in this study was mediated by increases in the amount of these cytokines. To this end, we first determined TNF-α and IL-6 levels in mouse livers. RT-PCR analyses showed that mRNA levels of hepatic TNF-α and IL-6 in obese mice were comparable to those in the control mice (data not shown). In addition, there was no significant difference in TNF-α levels between control and obese mice (Fig. 7A). Consistently, the amount of nuclear p65, a subunit of NF-κB, which is activated by TNF-α (32), was unchanged by obesity (Fig. 7B). For IL-6, a slight increase was observed in obese mice (Fig. 7A), but the extent of elevation was not strong enough to trigger the nuclear translocation of STAT3, a signal transducer of IL-6 (32), because no difference in nuclear STAT3 levels in the liver was observed between the groups (Fig. 7B).

Changes in Hepatic P450s in GTG-Induced Obese Mice

Because the expression profile of hepatic P450s in obese mice fed a high-fat diet was surprisingly different from those

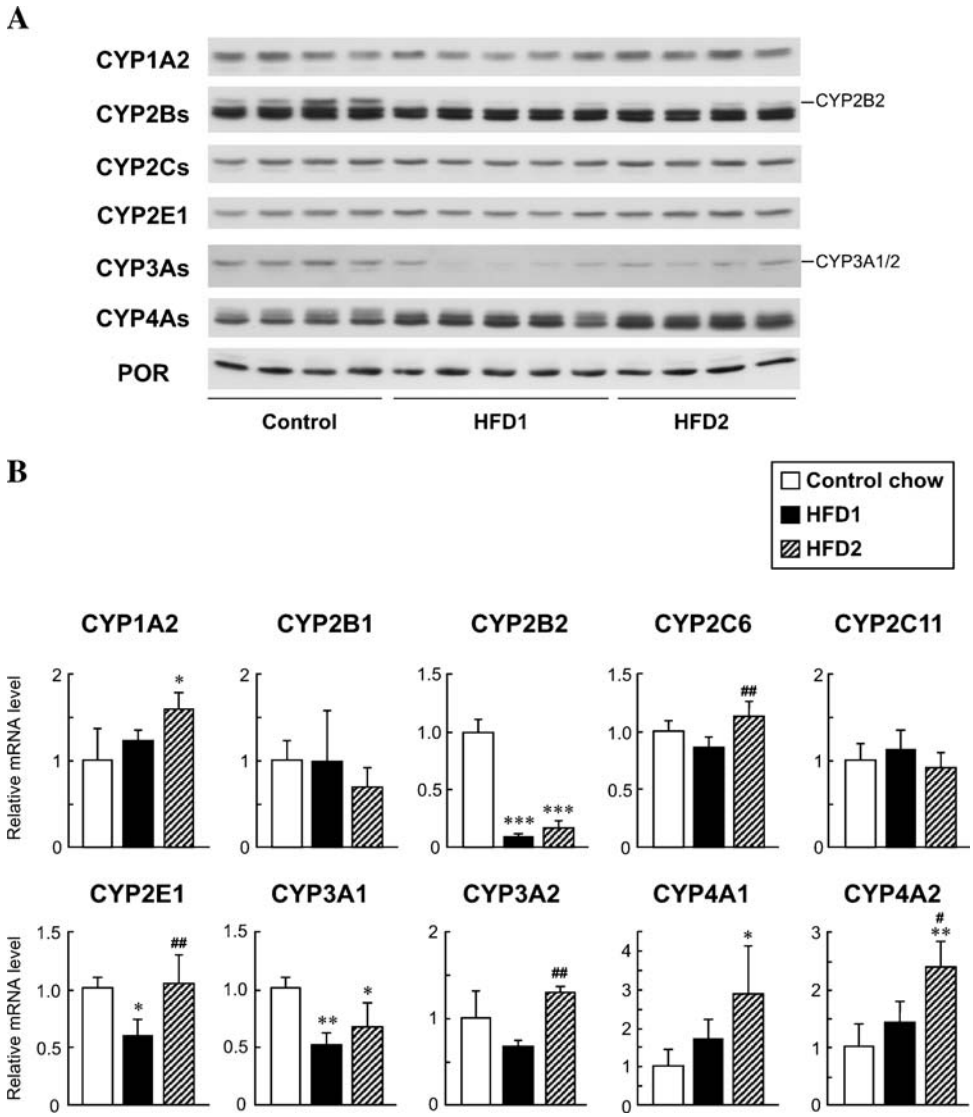

Fig. 10. Effects of 1-week administration of a high-fat diet on the expression of hepatic P450s in rats. Rats were fed control chow or a high-fat diet, HFD1 or HFD2, for 1 week. P450 protein (A) and mRNA (B) levels were determined by Western blotting (5 μg/lane) and quantitative RT-PCR, respectively, as described in the legend to Fig. 1. P450 mRNA levels were normalized with that of RPS9, and those of control mice were set at 1. Data represent the means ± SD (n = 4 or 5). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ *vs.* corresponding control mice. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ *vs.* mice fed HFD1.

of genetically obese animals such as Zucker rats, *db/db* and *ob/ob* mice (5–8,12), we employed another obese model, GTG-induced obese mice, to investigate whether the administration of a high-fat diet was associated with these changes. A single injection of GTG is known to induce ventromedial hypothalamic lesions, resulting in hyperphagia and obesity (33,34). In this study, 5-week-old male ICR mice were treated with GTG (300 mg/kg) or saline (vehicle) intraperitoneally and kept on regular chow for 4 weeks. GTG-treated mice consumed 1.32 times more food than saline-treated mice and were 1.34-fold heavier than control mice with a substantial increase in adipose tissue size (Table IV). Whereas there were no significant differences in serum lipids and glucose, obese mice accumulated more lipids in the liver than control animals (Table IV).

To investigate changes in the expression of hepatic P450s, quantitative RT-PCR experiments were carried out. As depicted in Fig. 8A, GTG-treated obese mice showed marked differences in expression compared with obese mice fed a high-fat diet. CYP3A11 and CYP2C29 mRNA levels were not affected by GTG-induced obesity. In contrast, in obese mice, CYP1A2 and CYP2E1 mRNA levels were decreased, whereas CYP2B10 and CYP4A10 mRNAs were increased. Changes in P450 protein levels were consistent with those in mRNA levels. In obese mice, CYP2Bs (the band corresponding to CYP2B10 is indicated by an arrowhead) and CYP4As were increased, and CYP1A2 and CYP2E1 proteins fell (Fig. 8B).

Effects of the Short-Term Administration of a High-Fat Diet on Hepatic P450s in Mice

As CYP3A down-regulation was observed in high-fat diet- but not GTG-induced obese mice, the reduction can be associated with the administration of a high-fat diet. To investigate this possibility, mice were fed a high-fat diet for a week, which did not induce obesity, and changes in P450 mRNA levels were examined (Fig. 9). Feeding HFD1 or HD2 drastically reduced hepatic CYP3A11 mRNA levels in the liver. In addition, CYP2C29 mRNA levels were also decreased, although the degree of reduction was smaller than that of CYP3A11 mRNA. These results suggest that the administration of a high-fat diet, at least for a week, represses the basal expression of *CYP3As* in mouse liver. Interestingly, in HFD1- but not HFD2-fed mice, CYP2B10 mRNA levels were also decreased, which was not observed in obese mice fed HFD1 for 5 weeks.

Effects of the Short-Term Administration of a High-Fat Diet on Hepatic P450s in Rats

To investigate whether there was a species-difference in the high-fat diet-induced CYP3A down-regulation, rats were fed a high-fat diet (HFD1 or HFD2) for a week, and mRNA and protein levels of hepatic P450s were determined (Fig. 10). The amount of CYP3A1/2 proteins fell drastically in HFD1-fed mice and moderately in HFD2-fed mice, reflecting changes in the mRNA levels. In contrast to the results obtained with mice, a high-fat diet did not alter both mRNA and protein levels of rat CYP2Cs. Species differences were also observed in CYP2Bs and CYP4Bs. CYP2B2 protein and mRNA levels were decreased, and those of CYP4As increased in rats but not in mice. HFD2 increased CYP1A2 mRNA levels and HFD1 decreased those of CYP2E1, although no such changes were observed in the protein levels of CYP1A2 and CYP2E1. POR protein levels were unchanged by a high-fat diet. These results suggest that the administration of a high-fat diet reduces the basal expression of CYP3As in rats as well as in mice, although there were species differences in the changes of other P450 forms.

DISCUSSION

Obesity is considered a risk factor for metabolic syndrome or syndrome X. Because changes in physiological and/or pathophysiological conditions often affect the pharmacokinetics and pharmacodynamics of therapeutic drugs, the expression profile of drug-metabolizing enzymes in obese subjects might be different from that in slim individuals. Using nutritionally obese mice, we have demonstrated that obesity drastically decreased CYP3As in the liver and small intestine. This reduction was observed in mRNA and protein/enzymatic activity levels, suggesting the transcriptional repression of *CYP3A* genes. Interestingly, changes in the expression of hepatic P450s seem to vary with the etiology of obesity because obese mice induced by hypothalamic lesions, which were fed regular chow, showed a different expression profile from those in obese mice fed a high-fat diet.

A high-fat diet causes a variety of metabolic changes in the whole body, leading to obesity. In this study, we observed the substantial accumulation of triacylglycerol in the liver of obese mice fed HFD1 or HFD2 (Table III) and found negative correlations between CYP3A11 mRNA levels and hepatic triacylglycerol content in the obese mice ($r = -0.889$, $P < 0.0001$ for HFD1-fed mice; $r = -0.587$, $P < 0.05$ for HFD2-fed mice). In addition, the 1-week administration of a high-fat diet reduced mRNA levels of CYP3As in the liver of mice and rats (Figs. 9 and 10), in which significant accumulation of triacylglycerol was also observed even without obvious obesity (data not shown). There are a few reports on the relationships between lipid changes and the expression of CYP3As. In humans, the reduction of CYP3A proteins in nonalcoholic steatohepatitis was suggested by immunohistochemistry (35). Leclercq *et al.* (36) reported in ducks that fat accumulation in the liver was associated with a significant decrease in erythromycin *N*-demethylase activity, which is catalyzed by CYP3As. Taken together, the hepatic accumulation of triacylglycerol could be one reason for reduced CYP3A expression by a high-fat diet. However, the lipid accumulation of HFD2-induced obese mice was greater than that of HFD1-fed mice (Table III), although the extent of CYP3A reduction was more prominent in obese mice fed HFD1 than those fed HFD2 (Figs. 2 and 3). Moreover, the hepatic accumulation of lipids in GTG-induced obese mice was comparable to that in HFD1-induced obese mice (Tables III and IV), whereas GTG-induced obesity did not alter the expression of CYP3As (Fig. 8). These results raise the possibility that metabolic changes induced by a high-fat diet but not GTG result in the attenuation of *CYP3A* expression. Alternatively, a specific lipid molecule may

inhibit the expression of *CYP3As*. Intriguingly, feeding a high-fat diet for a day or two to mice modulated the hepatic expression of PGC-1α and PGC-1β (37), which are known to act as coactivators for various transcription factors including nuclear receptors.

In addition to a high-fat diet, we used GTG to make mice obese. GTG is known to cause ventromedial hypothalamic lesions, resulting in hyperphagia and eventually obesity (33,34). In this study, we found that these obese mice showed a completely different expression profile of hepatic P450s from that observed in obese mice fed a high-fat diet (Fig. 8). Neither CYP3As nor CYP2Cs were altered in GTG-treated mice, whereas a significant increase in CYP2Bs and CYP4As and decrease in CYP1A2 and CYP2E1 were observed. Interestingly, these changes observed in GTG-induced obese mice are similar to those reported in genetically obese *ob/ob* and *db/db* mice (7,8). Because *ob/ob* and *db/db* mice have defects in the leptin-mediated signal transduction pathway that controls appetite at the hypothalamus, the mechanism by which the expression of hepatic P450s in GTG-treated mice is changed may be similar to that in genetically obese mice. Although limited data are currently available as to how obesity affects P450 expression in human livers, the expression pattern in obese humans may be more similar to that in obese mice fed a high-fat diet than genetically and GTG-induced obese animals because most human obesity results from lifestyle-related reasons such as the excess intake of calories.

Nuclear receptors HNF-4α and PXR have been demonstrated to play important roles in the regulation of both human and rodent CYP3As. Miyata *et al.* (21) identified three footprint regions (termed 6βA-A, 6βA-B, and 6βA-C) in the proximal promoter of rat *CYP3A2*. Among them, 6βA-A was identified as a binding site for HNF-4α (21,23) and 6βA-B for PXR (20). In the case of human *CYP3A4*, HNF-4α binds to the distal promoter sequence and PXR binds to both proximal and distal promoters (22,26). Comparison of the *Cyp3a11* proximal promoter sequence with those of *CYP3A2* and *CYP3A4* has shown that the *Cyp3a11* promoter is more similar to the *CYP3A2* promoter than to the *CYP3A4*'s, having 6βA-A-, 6βA-B-, and 6βA-C-like sequences (data not shown), and we have observed binding of both HNF-4α and PXR to the *Cyp3a11* promoter DNA (data not shown). In addition, other members of the nuclear receptor superfamily, COUP-TFII and CAR, also bound to the mouse *Cyp3a11* promoter (data not shown) as shown in rat and human *CYP3As* (23–25). These results suggest that the aforementioned nuclear receptors are also involved in the transcription of mouse *Cyp3a11*. Because we recently demonstrated that the expression of CAR in obese Zucker rats, which lack the PB induction of CYP2Bs, is significantly lower than in control lean mice (6), we speculated that the expression of HNF-4α, PXR, or CAR was decreased in obese-mouse livers, leading to the down-regulation of *CYP3A* expression. Alternatively, the increased amount of COUP-TF, which was demonstrated to suppress rat *CYP3A1* expression (24), might reduce *CYP3A* expression in obese-mouse livers. However, this was not the case in nutritionally obese mice (Fig. 6). These results suggest that changes in the expression of these nuclear receptors play a minor, if any, role in the down-regulation of *CYP3As*.

PXR is also important for the expression of several phase 2 enzymes and drug transporters (3,17,18). Thus, if high-fat diet-induced obesity suppresses the PXR-mediated transcription of *CYP3A* genes, the expression of these genes can also be attenuated. RT-PCR analyses, however, demonstrated that the mRNA levels of phase 2 enzymes and transporters examined were unaltered by obesity (Fig. 3). Furthermore, after DEX treatment, CYP3A mRNA and protein levels in obese mice were comparable to those in the control mice (Fig. 5). These results indicate that the mechanism of PXR-dependent transcription remains functional in obese-mouse livers and support the hypothesis that PXR is less important in the reduction of constitutive expression of CYP3As.

Proinflammatory cytokines have been reported to reduce the expression of hepatic P450s (29–31). As obesity is associated with high levels of circulating TNF-α and IL-6 (27,28), this raised the possibility that the down-regulation of CYP3As observed in obese mice was mediated by those cytokines. However, this is unlikely because obese mice fed HFD1 showed similar levels of both TNF-α and IL-6 and no nuclear accumulation of STAT3 and NF-κB (Fig. 7). In addition, nonobese mice fed a high-fat diet for only 1 week demonstrated reduced CYP3A11 mRNA in the liver (Fig. 9). Recently, Cheng *et al.* (38) proposed that the lipopolysaccharide-mediated down-regulation of *CYP2C11*, *CYP3A2*, and *CYP2E1* results from the decreased DNA binding of HNF-1, HNF-3β, and HNF-4α and decreased nuclear protein level of HNF-4α. In this study, we did not observe the obesity-dependent decrease of mRNA and nuclear protein levels of HNF-1, HNF-3, and HNF-4α (Fig. 6 and Yoshinari *et al.*, unpublished data) in mice fed a high-fat diet. Furthermore, the role of HNF-1 and HNF-3 in the transcriptional regulation of *CYP3As* is ambiguous at present. Taken together, the down-regulation of CYP3As induced by a high-fat diet is probably mediated through a mechanism different from the proinflammatory cytokine-mediated pathway.

In conclusion, we examined the expression profile of P450s in obese mice fed a high-fat diet and found that CYP3A levels were significantly decreased. This down-regulation was relatively specific to CYP3As among phase 1 and 2 enzymes and drug transporters. Although the precise mechanism has not yet been identified, metabolic changes induced by a high-fat diet and/or accumulation of a certain lipid molecule may be associated with the down-regulation of *CYP3A* genes. We are currently studying the physiological and clinical significance of the obesity-associated CYP3A down-regulation *in vivo* especially in obese/diabetic humans.

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