Consumption of a High-Fat Diet during Pregnancy Changes the Expression of Cytochrome P450 in the Livers of Infant Male Mice

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It has been recently reported that the consumption of a high-fat diet during pregnancy exerts various effects on fetuses and newborn mice. The purpose of this study was to determine the effects of a high-fat diet during pregnancy on the expression of cytochrome P450 (CYP) in the livers of offspring. Mouse dams were fed a high-fat diet during pregnancy from the time of conception. After their birth, the newborn mice were fed a normal diet until 12 weeks of age. In the livers of the infant male mice that consumed a high-fat diet, the protein expression of CYP3A and CYP2C was decreased, and the protein expression of CYP1A and CYP2E was increased at 6 and 12 weeks of age. However, almost no changes were observed in the CYP proteins at 6 and 12 weeks of age in the livers of the infant female mice that consumed a high-fat diet. The amount of pregnane X receptor (PXR) translocated into the nucleus was reduced in the livers of infant male mice that consumed a high-fat diet. However, there was neither an increase in tumor necrosis factor-*α* or interleukin-1*β* nor a decrease in lithocholic acid. These data suggested that CYP3A and CYP2C might decrease as a result of the decrease in the amount of nuclear PXR in infant male mice that consumed a high-fat diet. The results of this study suggested that the consumption of a high-fat diet by pregnant mothers may be one explanation for individual differences in pharmacokinetics.

Key words high-fat diet; cytochrome P450; pregnane X receptor; pharmacokinetics

Studies have reported that consumption of a high-fat diet during pregnancy exerts various effects on offspring.[1–4] For example, rats born to mothers that were given a high-fat diet during pregnancy showed an increased number of galanin-producing brain cells (galanin is an orexigenic peptide in the brain), which leads to obesity at 10 weeks of age.[1] Additionally, the level of brain-derived neurotrophic factor in the brain decreases, which can lead to the development of neurodegenerative diseases such as Alzheimer's disease.[4]

Cytochrome P450 (CYP) is major drug-metabolizing enzyme that is known to metabolize approximately 70% of the drugs used in clinical practice.[5] The role of the CYP3A subfamily is important because approximately 50% of all drugs are metabolized by CYP3A.[5] In humans, the expression level of CYP3A accounts for more than 30% and 70% of total CYP protein expression in the liver and the intestine, respectively.[6] Therefore, changes in CYP3A expression and activity exert significant effects on drug therapy.[7–9] It has been reported that there are sex differences in the expression of CYP. For example, in mice, CYP2D9 and CYP4A12 are specifically expressed in males, and CYP2A4, CYP2B9, CYP3A41, and CYP3A44 are specifically expressed in females.[10–12]

There have been many reports suggesting that the consumption of a high-fat diet differentially affects the expression and activity of CYP.[13,14] It has also been reported that obesity in mothers affects the expression of CYP1A1 in fetuses.[15] We have demonstrated that the expression and activity of CYP3A was decreased in male offspring when a high-fat diet was consumed during pregnancy.[16] However, it was unknown whether there was an influence on CYP species other than CYP3A or a difference between males and females in the expression of CYP in the offspring born to mothers that consumed a high-fat diet. In this study, we examined the effects of high-fat diet consumption by mothers during pregnancy on the expression of CYP species in the livers of offspring. Mouse dams were fed a high-fat diet during pregnancy from the time of conception. After their birth, the newborn mice were fed a normal diet until 12 weeks of age. The expression of CYP3A, CYP1A, CYP2C, CYP2D, and CYP2E in the livers was examined at 6 and 12 weeks of age. The mechanism responsible for the change in the expression of CYP3A and CYP2C was also examined.

MATERIALS AND METHODS

Materials Mouse anti-rat CYP2C6 antibody, bovine serum albumin (BSA), TRI reagent, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, U.S.A.). Rabbit anti-rat CYP3A2 antibody and goat anti-rat CYP2E1 antibody were purchased from Daiichi Pure Chemicals (Tokyo, Japan). Goat anti-mouse CYP1A2 antibody, goat anti-mouse pregnane X receptor (PXR) antibody, goat anti-mouse constitutive androstance receptor (CAR) antibody, and donkey anti-goat immunoglobulin G-horseradish peroxidase (IgG-HRP) antibody were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, U.S.A.). Rabbit anti-rat CYP2D6 antibody was purchased from Chemicon International Inc. (Temecula, CA, U.S.A.). Donkey anti-rabbit IgG-HRP antibody, sheep anti-mouse IgG-HRP

The authors declare no conflict of interest.
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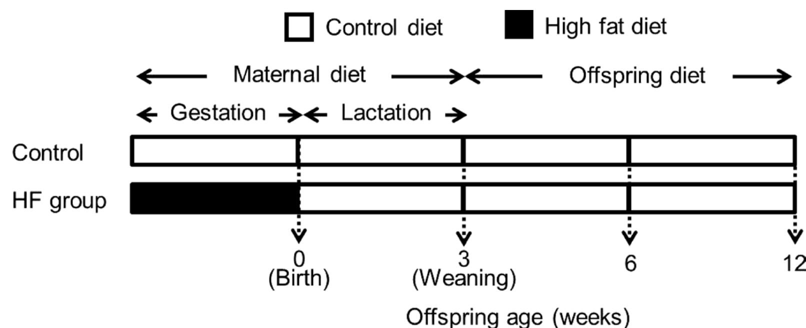

Fig. 1. Experimental Design

Table 1. Primer Sequences

Target	Accession number	Forward primer (5′→3′)	Reverse primer (5′→3′)	Amplicon size (bp)
TNF-α	NM_013693	AAGCCTGTAGCCCACGTCGTA	GGCGCCACTAGTTGGTTGTCTTTG	122
IL-1β	NM_008361	TGGCCTTGGGCCTCAAAG	GCTTGGGATCCACACTCTC	93
β-Actin	NM_007393	GAGCGCAAGTACTCTGTGTG	CGGACTCATCGTACTCCTG	86

antibody, and an enhanced chemiluminescence system (ECL) plus Western blotting detection reagents were purchased from GE Healthcare (Chalfont, St. Giles, U.K.). A NE-PER nuclear extraction kit was purchased from Thermo Fisher Scientific (Waltham, MA, U.S.A.). A QIAamp DNA stool mini kit was purchased from Qiagen Inc. (Valencia, CA, U.S.A.). A high capacity cDNA synthesis kit was purchased from Applied Biosystems (Foster City, CA, U.S.A.), and an iQ SYBR Green Supermix was purchased from Bio-Rad Laboratories (Hercules, CA, U.S.A.). Primers were purchased from Invitrogen Corp. (Tokyo, Japan). All other reagents were of the highest commercially available grade.

Animals ICR mice (8–10 weeks old) in their first day of pregnancy were purchased from Japan SLC, Inc. (Shizuoka, Japan). Mice were kept at room temperature (24±1°C) and 55±5% humidity with 12 h of light (artificial illumination: 08:00–20:00). The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, as adopted by the Committee on Animal Research at Hoshi University.

Experiment Pregnant ICR mice were given either the control diet (D12450B, Research Diets Corporation, New Brunswick, U.S.A.), which was a purified diet containing 10% of fat (lard), or the high-fat diet (D12492, Research Diets Corporation), which was a purified diet containing 60% of fat, throughout the pregnancy. All of the newborn mice were given the control diet during the suckling period and after weaning (Fig. 1). In this study, the liver was removed from the male and female offspring mice at either 6 or 12 weeks of age as well as from the mothers immediately after delivery, and the expression of CYP3A, CYP1A, CYP2C, CYP2D, and CYP2E was analyzed by Western blotting.

Blood Analysis Whole blood was collected using a heparinized syringe from the abdominal aorta of infant male and female mice at 6 and at 12 weeks of age as well as the mothers immediately after delivery under anesthesia with ethyl ether. Blood samples were centrifuged (1000×g for 15 min at 4°C), and the plasma was stored at −80°C until the assays were performed. Plasma glucose concentrations, triglyceride concentrations, total cholesterol concentrations, and free fatty acid concentrations were enzymatically quantified using a Glucose CII Test (Wako Pure Chemical Industries, Ltd., Osaka, Japan), Triglyceride E-Test Wako (Wako Pure Chemical), Cholesterol E-Test Wako (Wako Pure Chemical), and NEFA C-Test Wako (Wako Pure Chemical), respectively.

RNA Preparation from Tissue Samples RNA was extracted from approximately 15 mg of frozen liver tissue using TRI reagent. The resulting solution was diluted 50-fold using Tris/EDTA buffer (TE buffer), and the purity and concentration (μg/mL) of RNA were calculated by measuring the absorbance at 260 and 280 nm using a U-2800 spectrophotometer (Hitachi High Technologies, Tokyo, Japan).

Measurement of Liver Triglyceride Content Liver triglyceride content was measured as described previously.[17,18] Briefly, a portion (100 mg) of liver tissue was homogenized in phosphate buffer saline (pH 7.4, 1 mL). The homogenate (0.2 mL) was extracted with isopropyl alcohol (1 mL), and the extract was analyzed using a Triglyceride E-Test to determine liver triglyceride content.

Real-Time Polymerase Chain Reaction (PCR) A high-capacity cDNA synthesis kit was used to synthesize cDNA from 1 μg of RNA. TE buffer was used to dilute the cDNA 20-fold to prepare the cDNA TE buffer solution. The expression of target genes was detected using the primers in Table 1 for real-time PCR. To each well of a 96-well PCR plate, the following were added: 25 μL of iQ SYBR Green Supermix, 3 μL of forward primer of the target gene (5 pmol/μL), 3 μL of reverse primer (5 pmol/μL), 4 μL of cDNA TE buffer solution, and 15 μL of RNase-free water. The cycling used a denaturation temperature of 95°C for 15 s, an annealing temperature of 56°C for 30 s, and an elongation temperature of 72°C for 30 s. The fluorescence intensity of the amplification process was monitored using the My iQ™ single-color real-time RT-PCR detection system (Bio-Rad Laboratories). The mRNA levels were normalized against β-actin.

Microsome Preparation Approximately 100 mg of liver was homogenized with dissecting buffer (0.3 M sucrose, 25 mM imidazole, 1 mM EDTA, 8.5 μM leupeptin, and 1 μM

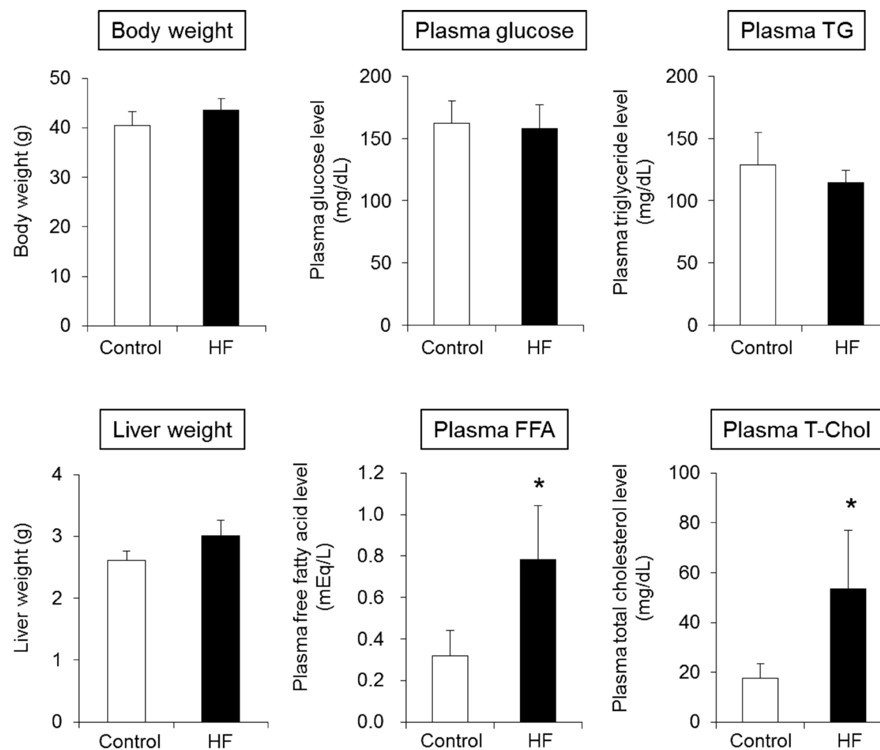

Fig. 2. Immediate Post-Delivery Body Weight, Liver Weight, and Biochemistry of Mouse Mothers That Consumed a High-Fat Diet during Pregnancy

Body weight, liver weight, and biochemistry were measured in mothers that had been fed either a normal diet (control) or a high-fat diet (HF group) during pregnancy. Data show the mean±S.D. of six mice per group. Student's *t*-test: $*p<0.05$ *vs.* control group. TG: triglyceride, FFA: free fatty acid, T-Chol: total cholesterol.

phenylmethylsulfonyl fluoride; pH 7.2). The resulting suspension was centrifuged (9000×*g* for 15 min at 4°C), and the supernatant was further centrifuged (105000×*g* for 1 h at 4°C). Dissecting buffer was added to the precipitate and homogenized using an ultrasonic homogenizer (UH-50, SMT Co., Ltd., Tokyo, Japan) to yield the microsomal fraction.

Nuclear Extraction Nuclear protein extract was prepared to examine the nuclear translocation of PXR and CAR. Protein was extracted according to the protocol of the NE-PER nuclear extraction kit. CER I solution was added to approximately 100 mg of the liver, followed by homogenization with a Teflon homogenizer and incubation on ice. CER II solution was added to the homogenate followed by centrifugation (16000×*g* for 5 min at 4°C) to remove the supernatant. NER solution was added to the precipitate, and after incubation, it was centrifuged (16000×*g* for 10 min at 4°C) to obtain the supernatant to be designated as the nuclear protein extract.

Electrophoresis and Immunoblotting Protein concentrations were measured by the BCA method[19] using BSA as a standard. Electrophoresis was performed using Laemmli's method.[20] Proteins were diluted 2-fold using loading buffer (84 mM Tris, 20% glycerol, 0.004% bromophenol blue, 4.6% sodium dodecyl sulfate, and 10% 2-mercaptoethanol; pH 6.8), and the samples were boiled for 5 min prior to loading on a polyacrylamide gel. After electrophoresis, the isolated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane, which was incubated in 1% skim milk blocking buffer for 1 h. After blocking, the membrane was incubated with primary antibodies for 1 h at room temperature. The following primary antibodies were used: rabbit anti-rat CYP3A2 (1/10000), goat anti-mouse CYP1A2 (1/500), mouse anti-rat CYP2C6 (1/1000), rabbit anti-rat CYP2D6 (1/25000), goat anti-rat CYP2E1 (1/35000), goat anti-mouse PXR (1/1000), and goat anti-mouse CAR (1/1000). After washing the membrane with TBS-Tween (20 mM Tris–HCl, 137 mM NaCl, and 0.1% Tween 20; pH 7.6), the membrane was incubated with secondary antibodies for 1 h at room temperature. After washing the membrane, the membrane was incubated with the ECL plus detection reagent and visualized with an LAS-3000 Mini Lumino image analyzer (FUJIFILM, Tokyo, Japan).

Quantification of Lithocholic Acid-Producing Bacteria DNA was extracted from approximately 200 mg of frozen feces using the QIAamp DNA stool mini kit. DNA extraction was carried out according to the protocol for the QIAamp DNA stool mini kit. The resulting solution was diluted 50-fold using TE buffer, and purity was confirmed and DNA concentration (μg/mL) was calculated by measuring absorbance at 260 and 280 nm using a spectrophotometer.

The expression of intestinal flora was detected by preparing primers and performing real-time PCR. To each well of a 96-well plate, 25 μL of iQ SYBR Green Supermix, 3 μL of forward primer (5 pmol/μL), 3 μL of reverse primer (5 pmol/μL), 2 μL of DNA TE buffer solution, and 17 μL of RNase-free water were added. For the cycling, the denaturation temperature was set at 95°C for 30 s, the annealing temperature at 60°C for 30 s, and the elongation temperature at 72°C for 1 min. The fluorescence intensity of the amplification process was monitored using the My iQ™ single-color real-time RT-PCR detection system. The following pairs of primers were used to detect the presence of *Bacteroides fragilis*: forward 5′-ctgaaccagccaagtagcg-3′ and reverse 5′-ccgcaaactttcacaactgactta-3′.

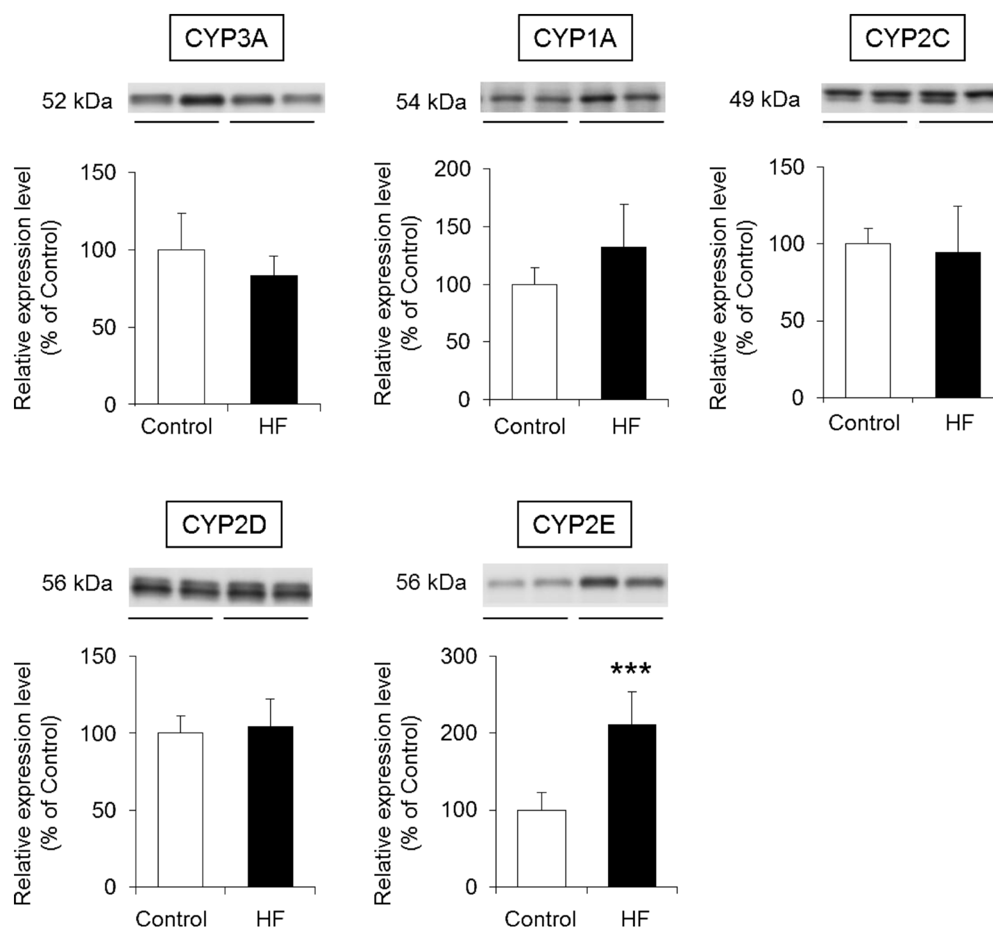

Fig. 3. Immediate Post-Delivery Expression of CYP Species in Mouse Mothers That Consumed a High-Fat Diet during Pregnancy

The microsome fraction was prepared from the livers of mothers that were fed either a normal diet (control) or a high-fat diet (HF group), during pregnancy. The protein expression of CYP3A, CYP1A, CYP2C, CYP2D, and CYP2E was analyzed by Western blotting. Data show the mean±S.D. of six mice per group. Student's *t*-test: *** $p<0.001$ *vs.* control group.

Statistical Analysis Numerical data are expressed as the mean±standard deviation. The significance of the differences was examined using Student's *t*-test. $p<0.05$ was considered to be significant.

RESULTS

The Effect of High-Fat Diet Consumption during Pregnancy on Mouse Mothers Immediately after Delivery The effect of the consumption of a high-fat diet during pregnancy on body weight, liver weight, blood biochemistry, and the expression of CYP species in the liver was examined in mouse mothers immediately after delivery (Figs. 2, 3). The body weight and liver weight of the mothers that consumed a high-fat diet (HF group) were not different from that of the control group. Moreover, no differences were observed in both the plasma glucose and triglyceride levels between the two groups. However, it was observed that the plasma total cholesterol and free fatty acid in the HF group increased compared to the control group (Fig. 2). The results were relatively consistent with the results from normal adult mice that were fed a high-fat diet.[21,22)]

Concerning the expression of CYP species in the liver, the expression of CYP3A, CYP1A, CYP2C, and CYP2D in the mothers that consumed a high-fat diet during pregnancy was not different from that in the control group, but the expression of CYP2E in the HF group was increased compared to the control group (Fig. 3). This increase in CYP2E was also consistent with the findings in normal adult mice that were fed a high-fat diet.[23)]

The Effect of High-Fat Diet Consumption during Pregnancy on the Change in the Expression of CYP Species in the Livers of Infant Male Mice We have previously reported that the CYP3A11 mRNA and CYP3A protein expression in the livers of newborn male mice born to the mothers that were given a high-fat diet during pregnancy was significantly decreased compared to the control.[16)] In this study, the effect on CYP species other than CYP3A was examined in the livers of newborn males when a high-fat diet was consumed during pregnancy (Fig. 4).

The protein expression levels of CYP1A in the liver at 6 weeks of age was higher in the HF group compared to the control group by approximately 5.4-fold. The expression of CYP2C in the HF group was approximately 30% of the level observed the control group. The expression of CYP2D in the HF group was not different from that in the control group. The expression of CYP2E in the HF group was approximately 1.8-fold that in the control group (Fig. 4A).

The protein expression of CYP1A in the livers at 12 weeks of age was approximately 1.9-fold higher in the HF group than the control group. The expression of CYP2C in the HF group was approximately 16% of the level observed in the control

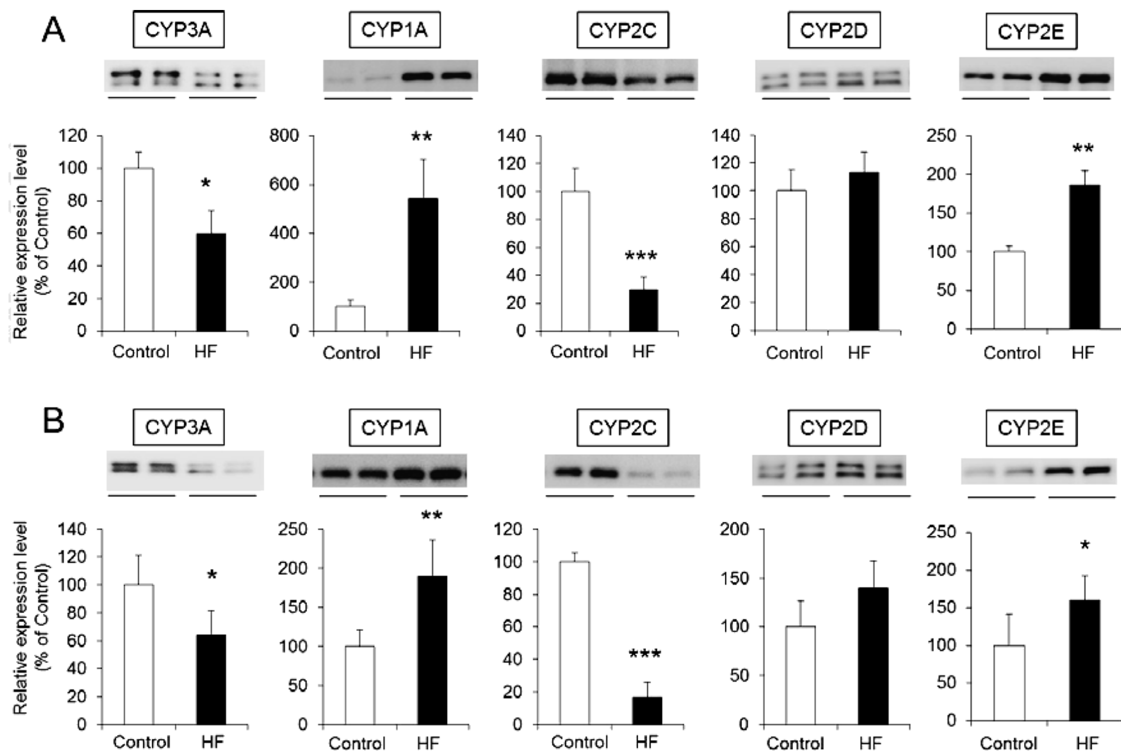

Fig. 4. The Effect of High-Fat Diet Consumption during Pregnancy on the Change in the Expression of CYP Species in the Livers of Infant Male Mice at 6 Weeks (A) and 12 Weeks (B) of Age

Mothers were fed either a normal diet (control) or a high-fat diet (HF group) during pregnancy. The microsome fraction was prepared from the livers of infant male mice at 6 weeks and 12 weeks of age, respectively, and the protein expression of CYP3A, CYP1A, CYP2C, CYP2D, and CYP2E was analyzed by Western blotting. Data show the mean±S.D. of six mice per group. Student's *t*-test: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ *vs.* control group.

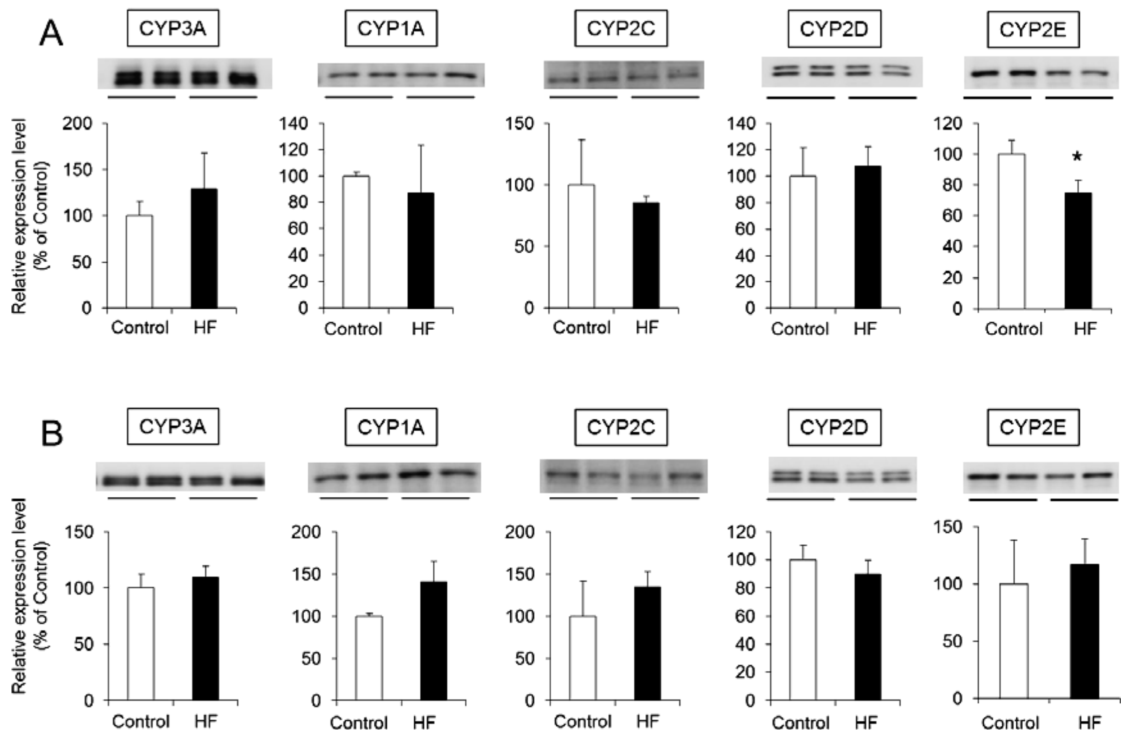

Fig. 5. The Effect of High-Fat Diet Consumption during Pregnancy on the Change in the Expression of CYP Species in the Livers of Infant Female Mice at 6 Weeks (A) and 12 Weeks (B) of Age

Mothers were fed either a normal diet (control) or a high-fat diet (HF group) during pregnancy. The microsome fraction was prepared from the livers of infant female mice at 6 weeks and 12 weeks of age, respectively, and the protein expression of CYP3A, CYP1A, CYP2C, CYP2D, and CYP2E was analyzed by Western blotting. Data show the mean±S.D. of six mice per group. Student's *t*-test: *$p<0.05$ *vs.* control group.

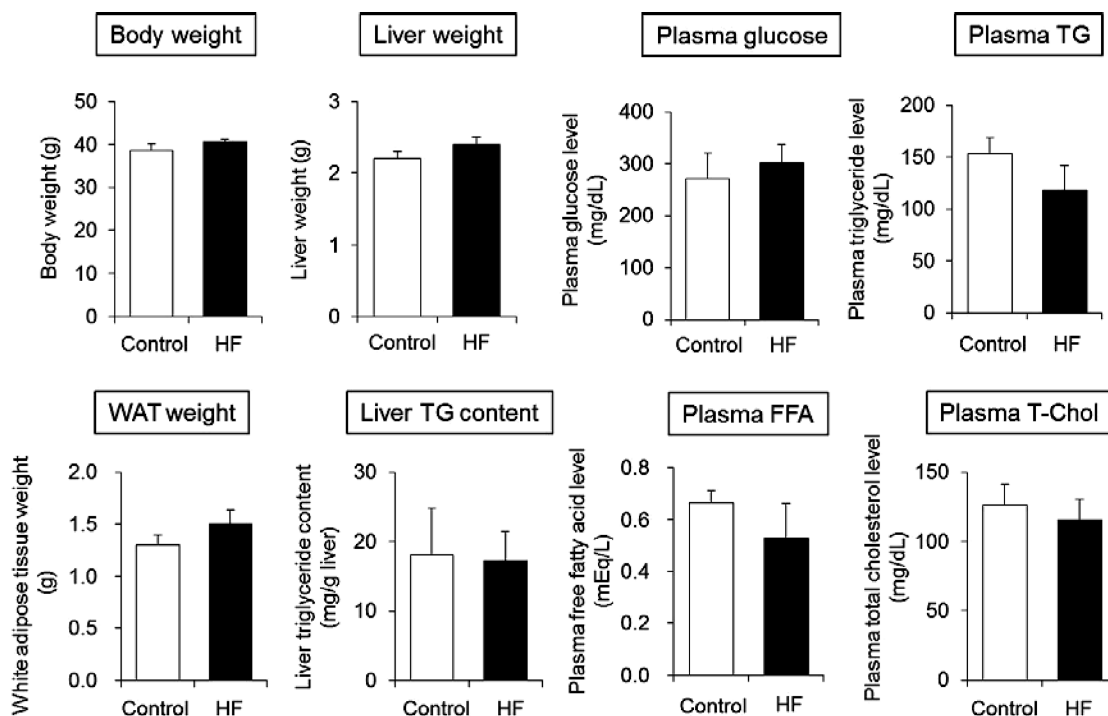

Fig. 6. The Effect of High-Fat Diet Consumption during Pregnancy on Body Weight, Liver Weight, Liver Triglyceride, White Adipose Tissue Weight, and Biochemistry in Infant Male Mice

Mothers were fed either a normal diet (control) or a high-fat diet (HF group) during pregnancy. The body weight, liver weight, liver triglyceride, white adipose tissue weight, and biochemistry of infant male mice at 6 weeks of age were measured. Data show the mean±S.D. of six mice per group. WAT: white adipose tissue, TG: triglyceride, FFA: free fatty acid, T-Chol: total cholesterol.

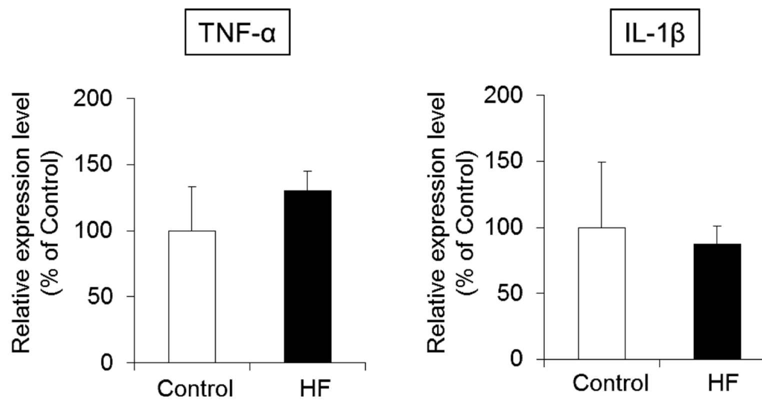

Fig. 7. The Effect of High-Fat Diet Consumption during Pregnancy on the Inflammatory Cytokine Levels in the Livers of Infant Male Mice

Mothers were fed either a normal diet (control) or a high-fat diet (HF group) during pregnancy. TNF-α and IL-1β mRNA expression levels in the livers of infant male mice at 6 weeks of age were measured by real-time RT-PCR, normalized to β-actin, and presented using the mean of the control group as 100%. Data show the mean±S.D. of six mice per group.

group. The expression of CYP2D in the HF group was not different from that in the control group. The expression of CYP2E in the HF group was approximately 1.6-fold that in the control group (Fig. 4B).

The expression levels of most CYP proteins measured in the HF group were different from those in the control group at both 6 and 12 weeks of age, and the pattern of change was similar between 6 and 12 weeks of age.

The Effect of High-Fat Diet Consumption during Pregnancy on the Change in the Expression of CYP Species in the Livers of Infant Female Mice The change in the expression of various CYP proteins observed in the livers of infant males was also examined in infant females (Fig. 5).

There were no differences in the protein expression of CYP3A, CYP1A, CYP2C, and CYP2D in the livers at 6 weeks of age between the control group and the HF group. However, the protein expression of CYP2E in the HF group was significantly reduced compared to the control (Fig. 5A).

There were no changes in the protein expression of CYP3A, CYP1A, CYP2C, and CYP2D in the livers at 12 weeks of age, but the reduction in CYP2E observed in the HF group at 6 weeks of age was not observed at 12 weeks of age (Fig. 5B).

Thus, it was observed that the consumption of a high-fat diet during pregnancy significantly changed the expression of CYP in newborn and infant males, but it had a less pronounced effect on infant females.

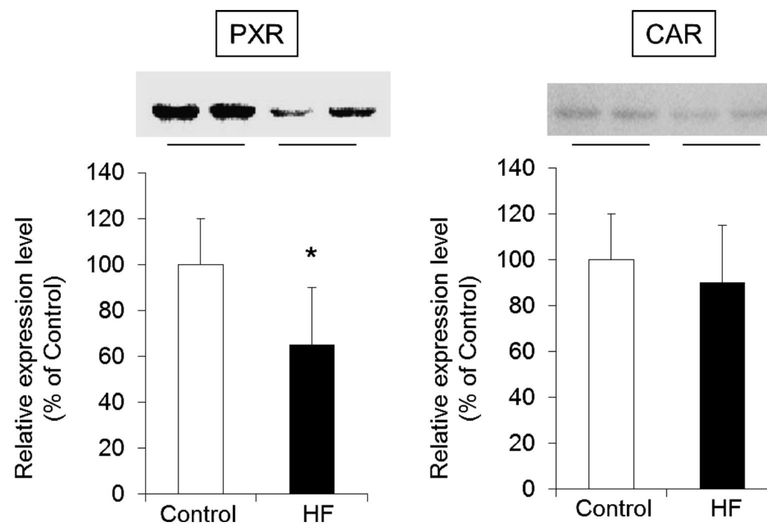

Fig. 8. The Effect of High-Fat Diet Consumption during Pregnancy on the Nuclear Translocation of PXR and CAR in the Livers of Infant Male Mice

Mothers were fed either a normal diet (control) or a high-fat diet (HF group) during pregnancy. PXR and CAR expression levels in the livers of infant male mice at 6 weeks of age were measured by Western blotting. Data show the mean±S.D. of six mice per group. Student's *t*-test: $^*p<0.05$ *vs.* control group.

The Effect of High-Fat Diet Consumption during Pregnancy on Body Weight, Liver Weight, Liver Triglyceride, White Fat Weight, and Biochemistry in Infant Male Mice Body weight, liver weight, white adipose tissue weight (around the testes, retroperitoneum, and kidney), liver triglyceride, and biochemistry were measured in infant mice born to mothers that were given a high-fat diet during pregnancy and given a normal diet after birth until 6 weeks of age (HF group). There were no differences between the control group and the HF group in body weight, liver weight, white adipose tissue weight, or liver triglyceride. No differences were observed between the control group and the HF group in blood glucose, triglyceride, total cholesterol, or free fatty acid (Fig. 6).

The Effect of High-Fat Diet Consumption during Pregnancy on the Inflammatory Cytokine Level in the Livers of Infant Male Mice The mRNA expression levels of the inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β were measured in the livers of infant male mice at 6 weeks of age (HF group). There were no differences observed in the mRNA levels of TNF-α and IL-1β at 6 weeks of postnatal age between the control group and the HF group (Fig. 7).

The Effect of High-Fat Diet Consumption during Pregnancy on the Nuclear Translocation of PXR and CAR in the Livers of Infant Male Mice The nuclear translocation of the nuclear receptors PXR and CAR was examined in the livers of infant male mice at 6 weeks of age. The nuclear translocation of PXR was significantly lower in the HF group compared to the control group. However, the nuclear translocation of CAR was not different between the control group and the HF group (Fig. 8).

The Effect of High-Fat Diet Consumption during Pregnancy on Enteric Bacteria in Infant Male Mice We have previously reported that an increase in lithocholic acid (LCA) produced by enteric bacteria contributes to an increase in the expression of CYP3A in the livers.[24,25] Therefore, we examined the amount of enteric bacteria (*Bacteroides fragilis*) that produce LCA in the large intestines of infant males born to mothers that consumed a high-fat diet during pregnancy. As a result, no differences were observed in the amount of enteric bacteria between the control group and the HF group (Fig. 9).

DISCUSSION

It is known that the nutrient balance in the mother during pregnancy affects the growth of the child after birth.[26,27] We have previously reported that when mothers consumed a high-fat diet during pregnancy (HF group), the CYP3A11 mRNA and CYP3A protein expression levels in the livers of infant male mice born to them were significantly decreased compared to the control, and that the decrease was observed immediately after birth and remained until 12 weeks after birth.[16]

In this study, the change in the expression of CYP species in the livers of infant males, infant females, and mothers immediately after delivery was examined when the mothers consumed a high-fat diet. As a result, there was a change in the expression of CYP2E in the livers immediately after delivery in mothers that had consumed a high-fat diet during pregnancy, but no changes were observed in CYP3A, CYP1A, CYP2C, and CYP2D (Fig. 3). However, in the livers of infant male mice in the HF group, a decrease in CYP3A and CYP2C as well as an increase in CYP1A were observed both at 6 and 12 weeks of age (Figs. 4, 10). In the livers of infant female mice, there were virtually no differences observed in the expression of various CYP species between the HF group and the control group (Figs. 5, 10). These data showed that the CYP expression pattern due to the consumption of a high-fat diet was different between mothers and their infant offspring. It was also observed that the expression pattern of CYP proteins was different between infant males and females born from these mothers.

Why did the expression of CYP3A and CYP2C decrease in the livers of infant males due to the consumption of a high-fat diet during pregnancy? It is generally known that the continuous consumption of a high-fat diet induces obesity and diabetes. It has also been reported that the activity of CYP3A4 in the liver decreases in cases of obesity and diabetes.[28,29] In this study, the caloric intake of the mothers during pregnancy was approximately 11 kcal/mouse/d and 15 kcal/mouse/d in the

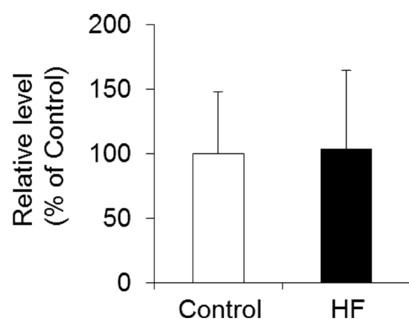

Fig. 9. The Effect of High-Fat Diet Consumption during Pregnancy on Enteric Bacteria in Infant Male Mice

Mothers were fed either a normal diet (control) or a high-fat diet (HF group) during pregnancy. The amount of DNA of *Bacteroides fragilis* in the feces of mice of infant male mice at 6 weeks of age was measured using real-time PCR. Data show the mean±S.D. of six mice per group.

Male 6 w	Male 12 w	CYP	Female 6 w	Female 12 w
⇩	⇩	3A	→	→
⇧	⇧	1A	→	→
⇩	⇩	2C	→	→
→	→	2D	→	→
⇧	⇧	2E	⇩	→

Fig. 10. Summary of the Expression of CYP Species in Infant Male and Female Mice by High-Fat Diet Consumption during Pregnancy

control group and the HF group, respectively. No differences were observed in the caloric intake after birth to 6 weeks of age (data not shown). As shown above, though there was a higher caloric intake during pregnancy in the HF group compared to the control group, no differences were observed between the control group and the HF group in body weight, liver weight, white adipose tissue weight or liver triglyceride in infant male mice at 6 weeks of age (Fig. 6). No differences were observed between the control group and the HF group with regards to blood glucose, triglyceride, total cholesterol, and free fatty acid (Fig. 6). Therefore, it is suggested that obesity and diabetes are excluded as the cause of the reduction in CYP3A in the livers in the HF group.

Inflammatory cytokines are one of the factors that affect the expression of CYP. It has been reported that the expression of CYP decreases when the amount of inflammatory cytokines increases.[30–33] Therefore, we measured the mRNA expression of inflammatory cytokines in the livers of infant males born to mothers that had consumed a high-fat diet. However, no changes in the TNF-α and IL-1β levels were observed between the control group and the HF group (Fig. 7).

The expression of CYP3A and CYP2C is controlled by the nuclear translocation level of the nuclear receptor PXR or CAR. It is known that when a ligand binds to either PXR or CAR. PXR or CAR is translocated from the cytoplasm into the nucleus and transcription is activated.[34–36] Therefore, the nuclear translocation of PXR and CAR was analyzed in the livers at 6 weeks of age. No differences were observed in the nuclear translocation of CAR in the livers between the control group and the HF group. However, the nuclear translocation of PXR in the HF group was reduced to approximately 70% of the level observed in the control group (Fig. 8). This suggested that the reduction in the expression of CYP3A and CYP2C may be due to reduction in the nuclear translocation of PXR.

LCA is one of the ligands of PXR. We have previously reported that the expression of CYP3A in the livers increases when there is an increase in LCA produced by enteric bacteria.[24,37] Therefore, the amount of enteric bacteria (*Bacteroides fragilis*) that produced LCA was measured in infant male mice in the HF group. No differences were observed in the amount of *Bacteroides fragilis* between the control group and the HF group (Fig. 9).

Based on the results described above, it was suggested that a reduction in the nuclear translocation of PXR may be the cause of reduction in CYP3A and CYP2C in the livers of infant male mice in the HF group. However, the reason why the nuclear translocation of PXR was reduced is still unknown. It is unlikely that a decrease in the PXR ligands such as LCA, vitamin E, and vitamin K triggers this phenomenon because the levels of these ligands are the same between the control group and the HF group (data not shown). Because a specific reduction in CYP3A and CYP2C was observed in the livers of infant male mice in the HF group, it is possible that the consumption of a high-fat diet affected the production of sex hormones such as testosterone and estradiol. However, no differences were observed in the production of the two hormones between the two groups in infant mice at 6 weeks of age (data not shown).

In summary, a reduction in CYP3A and CYP2C as well as an increase in CYP1A and CYP2E was observed in the livers of infant male mice born to mothers that consumed a high-fat diet during pregnancy. It was shown that these changes in expression did not return to the baseline until at least 12 weeks of age even though a normal diet was administered after birth (Fig. 10). It was also suggested that a reduction in the nuclear translocation of PXR was involved in the reduction in the expression of CYP3A and CYP2C in male infants. Future directions include investigating the mechanism responsible for the increase in CYP1A and CYP2E in the HF group. These results suggested that the consumption of a high-fat diet by pregnant mothers may be one of the reasons for individual differences in pharmacokinetics.

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