Diet-induced Lipid Accumulation in Liver Enhances ATP-binding Cassette Transporter g5/g8 Expression in Bile Canaliculi

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Summary: The ATP-binding cassette half-transporters Abcg5 and Abcg8 promote the secretion of neutral sterols into bile. Studies have demonstrated the diet-induced expression of these transporters in liver, but precisely where this occurs remains to be elucidated. This study investigated the changes in the expression of these transporters in bile canaliculi in cholesterol-loaded livers. Mice were fed either a standard (SD) diet or a high-fat and high-sucrose (HF/HS) diet for 3 weeks. Bile canaliculi proteins and cryosections were prepared from the liver, and the protein levels and distribution of Abcg5/Abcg8 were determined. The high-calorie diet induced a marked accumulation of lipids in mouse liver. Protein levels of Abcg5 and Abcg8 in bile canaliculi were significantly increased by the HF/HS diet compared to the SD diet. No significant differences in Abca1, Abcb4 (Mdr2), Abcb11 (Bsep), or Abcc2 (Mrp2) levels were observed. Immunohistochemical analyses confirmed that these increases occurred in bile canaliculi. These results suggest that diet-induced lipid loading of the liver causes a significant increase in the expression of Abcg5 and Abcg8 in bile canaliculi.

Keywords: Abcg5; Abcg8; bile canaliculi; high-calorie diet; Lxr

Introduction

The liver is a key organ in the maintenance of cholesterol homeostasis, showing active de novo cholesterologenesis and being able to transport impressive amounts of newly synthesized and diet-derived cholesterol via a number of distinct pathways. Cholesterol trafficking in the liver involves the concerted actions of a number of transporter proteins.1,2 In particular, several ATP-binding cassette (ABC) transporters fulfill crucial roles. For instance, the ABCG5/ABC8 (human)/Abcg5/Abcg8 (mouse, rat) couple is vital for hepatobiliary cholesterol excretion,3,5 while ABCA1 (human)/Abca1 (mouse, rat) is essential for high-density lipoprotein formation and, hence, for inter-organ trafficking of the highly water-insoluble cholesterol molecules.1,2

ABCG5 and ABCG8 (Abcg5 and Abcg8) are members of the large family of ABC transporters.6 They are half-transporters belonging to the G subfamily, which consist of two modules, i.e., a magnesium-dependent ATPase catalytic domain and a transmembrane domain containing six membrane-spanning segments.7 Based on results obtained in vivo and in vitro, these half-transporters are proposed to function as obligate heterodimers.8,9 The role of Abcg5 and Abcg8 in sterol trafficking in vivo has been examined in detail using genetically modified mice: biliary cholesterol secretion was markedly reduced in mice deficient in Abcg5, Abcg8, or both, whereas transgenic mice overexpressing human ABCG5 and ABCG8 had increased rates of fecal neutral sterol secretion.10-14 These findings suggest that Abcg5 and Abcg8 are located in liver bile canaliculi, but their exact distribution remains to be established because of an absence of antibodies for immunohistochemistry.

Studies indicate that dietary constituents perturb cholesterol homeostasis and consequently influence the expression of hepatic transporters. It is essential to study the effects of
dietary factors on the biological functions of hepatic transporters, because high-calorie diets can cause a variety of pathological conditions, e.g., cholesterol gallstone disease. Diets rich in cholesterol, cholic acid, or both are reported to enhance the expression of Abcg5/Abcg8 in liver.15-22 However, precisely where the increase occurs is unclear because of a lack of immunohistological data. Since these transporters function in bile canaliculi, verifying whether the increase in ABCG5/ABCG8 occurs in this region is quite important. To investigate changes in Abcg5/Abcg8 protein expression, we conducted an immunoblot analysis using newly raised antibodies and biochemically isolated bile canaliculi (the bile canaliculi fraction) and immunohistochemistry, we then compared mice fed a standard diet with those fed a high-calorie diet (high-fat and high-sucrose diet) known to cause marked increases in lipids in liver.23-26 The effect of the liver X receptor (LXR (human)/Lxr (mouse, rat)) on the expression of Abcg5/Abcg8 is also discussed.

**Materials and Methods**

**Antibodies:** Anti-CD13 (CD, cluster of differentiation) antibody was purchased from BMA Biomedicals (Augst, Switzerland); anti-CD31 antibody from BD Bioscience (San Jose, CA); horseradish peroxidase (HRP)-conjugated anti-β-actin antibody and anti-Abcb4 (Mdr2) antibody from Abcam (Cambridge, UK); and anti-Abcc2 (Mrp2) antibody, anti-Abcc3 (Mrp3) antibody, anti-Abcb11 (Bsep) antibody, and rhodamine-conjugated goat anti-rat antibody from Santa Cruz Biotechnology (Santa Cruz, CA). HRP-conjugated goat anti-rabbit IgG and HRP-conjugated rabbit anti-goat IgG were purchased from Vector Laboratories (Southfield, MI) and alexa488-conjugated goat anti-rabbit IgG from Invitrogen (Carlsbad, CA). The polyclonal rabbit anti-sera to mouse Abcg5 and Abcg8 peptides were developed in-house using a 15-peptide immunogen from mouse Abcg5 (1–15, MGELPFLSPEGARGC and a 14-peptide immunogen from mouse Abcg8 (1–14, MAEKTEETQLWNG), respectively.

**Experimental animals:** All the experiments followed protocols approved by the Institutional Animal Care and Use Committee, University of Shizuoka. Male C57BL/6 mice were obtained from Japan SLC (Hamamatsu, Japan) at 7 weeks of age and were acclimatized for 1 week before the experiments. The animals were housed in stainless-steel hanging cages with free access to food and water and were maintained on a 12-h light/dark cycle. The animals were randomly assigned to groups and given a standard (SD) diet or a high-fat/high-sucrose (HF/HS) diet for 3 weeks. The HF/HS 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); 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). Three times per week, all mice were weighed and the food intake in grams was monitored.

**Blood and tissue sampling:** Mice were anesthetized with diethyl ether, the abdominal cavity was opened, and blood was drawn from the abdominal vena cava into syringes. Serum samples were separated by centrifugation after incubation for 30 min at 37 °C. The resulting serum was stored at −30 °C until analysis. Livers were rapidly excised and weighed. The liver median lobe was excised and used for preparation of the membrane fraction and preparation for immunohistochemistry or for hematoxylin-Oil Red O staining.

**Preparation of the crude liver membrane fraction and the bile canaliculi-rich fraction:** Crude liver membranes were prepared as described by Ogawa.27 Liver was homogenized in 5 volumes of a homogenate buffer consisting of 0.1 M Tris-HCl buffer, pH 7.4, and 1 µg/ml leupeptin, 1 µg/ml peptatin A, and 50 µg/ml phenylmethylsulfonyl fluoride (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 homogenate buffer and was again centrifuged at 100,000 g for 30 min. The crude membrane fraction was resuspended in the homogenate buffer.

The bile canaliculi fraction was prepared according to a modification of the method described by Tsukita.28 The mouse liver was minced with razor blades and treated with a hypotonic solution consisting of 1 mM NaHCO3 and 1 µg/ml leupeptin, 1 µg/ml peptatin A, and 50 µg/ml PMSF for 45 min. The swollen samples were homogenized in 2 volumes of the hypotonic solution by the use of a loose-fitting homogenizer (Dounce, Vineland, NJ). The homogenate was diluted with up to 40 volumes of the hypotonic solution, filtered twice through four layers of gauze, and centrifuged at 1,500 g for 10 min. The pellets were resuspended in a hypotonic solution, diluted to 1.31 ml, and mixed with 9.69 ml of a 55% (w/v) sucrose solution to make a 48.55% sucrose solution. This was poured into a centrifuge tube and carefully layered with a 42.9% sucrose solution. After centrifugation for 60 min at 100,000 g, the bile canaliculi fraction was recovered at the 42.9:48.5% interface.

**Biochemical analysis:** Glucose, triacylglycerol, and total cholesterol levels in serum were measured enzymatically with kits from Shino Test (Tokyo, Japan). Frozen livers were homogenized in 20 volumes (the SD group) or 100 volumes (the HF/HS group) of phosphate-buffered saline (PBS) containing 0.1% Triton X-100; triacylglycerol, total-cholesterol, and free fatty acid concentrations were estimated.

**Immunoblot analysis:** Crude membrane fractions or bile canaliculi fractions were resolved on an SDS-7.5% polyacrylamide gel and then electroblotted onto a polyvinylidene difluoride membrane (Millipore Corporation, Bedford, MA). The immunoblots were consequently incubated with the primary antibodies at 4 °C overnight,
and with appropriate secondary antibodies conjugated with horseradish peroxidase at room temperature for 1 h. The antigen-antibody complexes were detected using an enhanced chemiluminescence system (GE Healthcare Biosciences, Piscataway, NJ).

**Immunohistochemistry:** For immunohistochemical analyses, 8-μm-thick frozen sections of mouse liver were air dried on glass slides and kept at −30°C until used. The sections were fixed by treatment with cooled methanol for 30 s at −30°C. After frozen sections were washed with PBS, nonspecific binding of antibodies was blocked with 5% normal goat serum and 2% bovine serum albumin (BSA). The samples were incubated with primary antibodies overnight at 4°C, and with secondary antibodies for 1 h at room temperature. Coverslips were mounted with 50% glycerol and slides of samples were subjected to microscopic observation. Images were acquired with a Zeiss LSM510 confocal microscope (Carl Zeiss, Oberkochen, Germany).

**Determination of mRNA levels:** Total RNA was prepared from the liver using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA). Samples were quantitated by spectrophotometry, and 1 μg of total RNA was used to generate cDNA by reverse transcription using a Prime Script II reverse transcription reagent kit (Takara, 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 using SYBR premix Ex Taq reagent (Takara) for the intercalation reaction with SYBR Green I according to the manufacturer’s specification. The thermal cycle conditions were as follows: hold for 40 cycles of 95°C for 30 s, then a two-step polymerase chain reaction using a Prime Script reverse transcription reagent kit (Takara, 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 using SYBR premix Ex Taq reagent (Takara) for the intercalation reaction with SYBR Green I according to the manufacturer’s specification. The respective forward and reverse primers for the genes were as follows: Abca1 (NM_013454), tagctggtctcagcagctc and attttaggacacttccggaaaac; Abcg5 (NM_031884), catccgtaaagacctctatgccaac and atggagccaccctgtggtcctacagcgtca and agatgcacataatctggccactctc; Abcg8 (NM_026180), tcagtccaacactctggaggtca and atttccggatgccatccgatcc; Lxr (NM_013839), ttagaatgtccagggctccag and tggtaatgtccagggctccag and tggtaatgtccagggctccag and tggtaatgtccagggctccag and tggtaatgtccagggc; Mrp3 (NM_019739), catccgtaaagacctctatgccaac and agatgcacataatctggccactctc; Abcc3 (NM_007393), catccgtaaagacctctatgccaac and agatgcacataatctggccactctc; β-actin (NM_007393), catccgtaaagacctctatgccaac and agatgcacataatctggccactctc.

Results

**Identification of Abcg5 and Abcg8 in bile canaliculi-rich membrane preparations of mouse liver:** To investigate the expression of hepatic transporters in bile canaliculi, we first tried to isolate bile canaliculi-rich fractions biochemically. Figure 1A shows an immunoblot analysis of the crude liver membrane fraction and the bile canaliculi fraction from mouse liver with antibodies against Abcc2 (Mrp2) and Abcc3 (Mrp3) which are located in the bile canaliculi and sinusoidal regions, respectively. The band corresponding to Abcc2 was detected only in the bile canaliculi fraction, whereas the band corresponding to Abcc3 was detected only in the crude liver membrane fraction, indicating the constituents of bile canaliculi to be abundant in the bile canaliculi fraction.

Peptide antibodies were raised against the N-terminus of mouse Abcg5 and mouse Abcg8 and were affinity purified prior to use. Western blotting showed that the anti-Abcg5 antibody bound to 79-, 73-, and 67-kDa proteins, the bands for which disappeared when the antibody was pre-incubated with the peptide against which it was raised (Fig. 1B). In the lane of the bile canaliculi fraction treated with PNGase F, only a 61-kDa band was detected (Fig. 1B). The 79-kDa band was intense in the bile canaliculi fraction, indicating that it corresponds to the mature form of Abcg5. On the other hand, the anti-Abcg8 antibody specifically bound to a 68-kDa protein, which was abolished by pre-treatment of the antibody with the specific peptide. The protein was abundant in the bile canaliculi fraction, and the band migrated to the 62-kDa position on treatment with PNGase F (Fig. 1B), indicating that the 68-kDa band corresponds to the mature form of Abcg8.

**Feeding of the HF/HS diet induced significant accumulation of lipids in mouse liver:** We previously reported that a HF/HS diet caused marked increases in liver and serum lipids, especially cholesterol in male rats. 21-26 Consistent with these observations, consumption of the HF/HS diet by male mice for 3 weeks resulted in major increases in absolute liver weight, relative liver weight, and liver lipids, including a marked accumulation of triacylglycerols, free fatty acids, and cholesterol (Table 1).

**Supplemental Figure 1** displays representative photomicrographs of liver sections stained with Oil Red-O. Numerous cytoplasmic lipid droplets were observed throughout the liver sections of HF/HS diet-fed mice, whereas those of mice fed an SD diet appeared normal, indicating the aberrant accumulation of lipids in livers of mice fed the HF/HS diet.

**Effect of HF/HS intake on expression of the hepatic transporters in mouse liver:** Using the bile canaliculi fractions prepared from mice fed the SD or the HF/HS diet, we checked the changes in the protein levels of hepatic transporters (Fig. 2). Interestingly, marked increases in Abcg5 and Abcg8 (6.6 and 4.7-fold, respectively) were observed in accordance with increases in their genes (Table 2). No significant differences in the levels of Abca1, Abcb4 (Mdr2), Abcb11 (Bsep), or Abcc2 were detected between the HF/HS diet- and the SD diet-fed mice. Western blot immunostaining for β-actin confirmed equivalent protein loading.
We further examined the changes in the distribution of Abcg5 and Abcg8 by conducting immunohistochemical analyses with frozen serial sections of mouse liver (Fig. 3). The distribution of the two proteins appeared to be similar not only histologically, but also at the cellular level. In the histological analysis, these transporters were mainly detected on plasma membranes of liver cells. To confirm the apical location of Abcg5 and Abcg8, immunohistochemical experiments were carried out using an antibody against a known apical marker, CD13, in the liver. As anticipated, these transporters were highly co-localized with CD13. Pre-immune sera showed no positive signals in bile canaliculi. In liver sections of mice fed the HF/HS diet, levels of Abcg5/Abcg8 in bile canaliculi were significantly increased. To examine whether the enhanced expression was restricted to the canaliculi, we further checked the co-localization of these transporters with CD31 as a sinusoidal tract marker. As shown in Figure 3, no overlapping was observed between CD31 and Abcg5/Abcg8. These observations indicate that the diet-induced accumulation of cholesterol in liver leads to enhanced expression of Abcg5/Abcg8 in a restricted region, i.e., bile canaliculi.

**Effect of a liver X receptor agonist on the expression of hepatic transporters:** Since the liver X receptor (LXR/Lxr) is reported to contribute to the protein expression of Abcg5/Abcg8,5,15,16,19,21,22 we compared the diet-induced expression of Abcg5/Abcg8 in bile canaliculi with expression of Abcg5/Abcg8 induced by TO901317, a synthetic agonist for LXR/Lxr. As reported previously,11 levels of Abca1 gene and protein expression in the crude membrane fractions from liver were elevated

### Table 1. Effect of the high-fat and high-sucrose diet on body weight, liver weight, serum lipids, and liver lipids

<table>
<thead>
<tr>
<th></th>
<th>SD</th>
<th>HF/HS</th>
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<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td>24.9 ± 0.6</td>
<td>24.7 ± 0.6</td>
</tr>
<tr>
<td><strong>Liver weight (g)</strong></td>
<td>1.3 ± 0.1</td>
<td>1.8 ± 0.1*</td>
</tr>
<tr>
<td><strong>Liver weight/body weight (%)</strong></td>
<td>5.3 ± 0.3</td>
<td>7.1 ± 0.4**</td>
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<tr>
<td><strong>Serum</strong></td>
<td></td>
<td></td>
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<tr>
<td>Triacylglycerol (mg/L)</td>
<td>7.5 ± 1.8</td>
<td>4.4 ± 1.3*</td>
</tr>
<tr>
<td>Free fatty acid (μEq/L)</td>
<td>455.7 ± 110.4</td>
<td>602.5 ± 91.1</td>
</tr>
<tr>
<td>Total cholesterol (mg/L)</td>
<td>9.2 ± 0.9</td>
<td>23.9 ± 6.1*</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triacylglycerol (mg/g liver)</td>
<td>12.9 ± 2.7</td>
<td>31.5 ± 11.0</td>
</tr>
<tr>
<td>Free fatty acid (μEq/g liver)</td>
<td>7.3 ± 0.5</td>
<td>14.2 ± 2.3**</td>
</tr>
<tr>
<td>Total cholesterol (mg/g liver)</td>
<td>5.3 ± 0.9</td>
<td>26.3 ± 9.8*</td>
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Values are mean ± S.D. of five mice.
*P < 0.05, **P < 0.005 versus SD diet-fed mice.

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Table 2. Hepatic gene expression of transporters and transcription factors in mice fed the high-calorie diet or administered Lxr agonist

<table>
<thead>
<tr>
<th>Gene</th>
<th>High-calorie diet (fold increase)</th>
<th>Lxr agonist (fold increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>HF/HS</td>
</tr>
<tr>
<td>Abcg5</td>
<td>1.00 ± 0.12</td>
<td>3.81 ± 0.62*</td>
</tr>
<tr>
<td>Abcg8</td>
<td>1.00 ± 0.09</td>
<td>3.93 ± 0.64*</td>
</tr>
<tr>
<td>Abcb4</td>
<td>1.00 ± 0.09</td>
<td>1.66 ± 0.23*</td>
</tr>
<tr>
<td>Abcb11</td>
<td>1.00 ± 0.09</td>
<td>0.68 ± 0.05*</td>
</tr>
<tr>
<td>Abcc2</td>
<td>1.00 ± 0.12</td>
<td>0.25 ± 0.06*</td>
</tr>
<tr>
<td>Abca1</td>
<td>1.00 ± 0.16</td>
<td>0.76 ± 0.16</td>
</tr>
<tr>
<td>Actin</td>
<td>1.00 ± 0.16</td>
<td>0.76 ± 0.16</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. of five (high-calorie diet), or three to four (Lxr agonist) mice.

*P < 0.05 versus SD diet-fed mice or vehicle-administered mice.

(Fig. 4 and Table 2), confirming the efficacy of this treatment. However, the increases in the protein levels of Abcg5 and Abcg8 in the bile canaliculi fraction (1.9- and 1.5-fold the vehicle-treated control level, respectively) (Fig. 4) were far less than those seen in mice fed the HF/HS diet, although the mRNA levels of Abcg5 and Abcg8 in the liver of the TO901317-administered mice were elevated as reported previously16,19,21,34 (both 2.2-fold the control level) (Table 2). Immunohistochemistry showed no significant increases in the expression of Abcg5/Abcg8 in bile canaliculi (Fig. 5 and Supplemental Fig. 2). Further, no up-regulation of Lxr, FoxO1, or Fxr expression, reported to induce the gene expression of Abcg5/Abcg8,16,18,32 was observed in the HF/HS diet-fed mice (Table 2). These observations suggest the nuclear receptors to be involved only partially or not at all in the diet-induced expression of Abcg5/Abcg8 in bile canaliculi.

Discussion

Here we established a system to examine changes in the expression of the Abcg5/Abcg8 proteins in bile canaliculi using newly developed antibodies. This study is the first to clarify the distribution of these transporters in liver based on physiological and biochemical data.

Since no antibodies against Abcg5 and Abcg8 were available for immunoblot analyses and immunohistochemistry, we produced antibodies against the N-terminus of mouse Abcg5 and Abcg8. Immunoblot analyses with biochemically isolated bile canaliculi, i.e., the bile canaliculi fraction, and deglycosylation-migration assays confirmed that the newly raised antibodies specifically recognized these transporters whether they were glycosylated or not (Fig. 1). Next, we confirmed that the antibodies are suitable for immunohistochemistry with frozen sections of mouse liver. Overall, the immunohistochemical profile of Abcg5 in liver was similar to that of Abcg8, with strong expression in bile canaliculi (Fig. 3). This result supports the heterodimerization of these proteins in bile canaliculi in the liver.8,9 Klett et al. demonstrated the distribution of ABCG5 and ABCG8 in human liver by immunohistochemistry.33 However, their data are inconsistent with ours in that: (1) the distribution pattern of ABCG5 was different from that of ABCG8 in liver, and (2) the signals corresponding to Abcg5 were also detected in the sinusoidal tracts. The antibodies raised by Klett et al. each detected only one protein band, which failed to migrate with glycosidase treatment, indicating the possibility that their antibodies only recognized the
Fig. 3. Effect of the HF/HS diet on the distribution of Abcg5 and Abcg8 in mouse liver
Mouse liver cryosections were stained with a combination of anti-Abcg5/CD13 or anti-Abcg5/CD31 antibodies (A), and anti-Abcg8/CD13 or anti-Abcg8/CD31 antibodies (B). The pre-immune controls for both antibodies are as marked and shown in the top right hand corner of each panel. The first row shows the immunological profiles of the liver from the SD diet-fed mice. The second and third rows indicate those from the HF/HS diet-fed mice. Antibodies against CD13 (second row) and CD31 (third row) were used as a marker for bile canaliculi and the sinusoidal tract, respectively. In the HF/HS diet-fed mice, the colocalization of Abcg5/Abcg8 and CD13 (indicated by arrows) is increased in liver. The graph on the right shows the expression of Abcg5 or Abcg8 in bile canaliculi. The ratio was calculated by dividing the merged area (yellow area) by the area of CD13 (red area) from photographs in which about a hundred parenchymal cells were present. Fold increases were estimated by taking the control values obtained from the SD diet-fed mice as 1. Each value is the mean ± S.E. for 40 specimens (3 mice).

*P < 0.05, **P < 0.01 for the HF/HS diet-fed mice versus the SD diet-fed mice. Bar = 10 µm.
unglycosidated form of the transporters. Since the mature form of both proteins is proposed to be glycosidated, this is the first report to confirm that the Abcg5/Abcg8 proteins are certainly located in bile canaliculi in liver.

Many studies have demonstrated that nutritional status has an influence on the expression of hepatic transporters. With regard to ABCG5 and ABCG8, diets rich in cholesterol, cholic acid, or both have been reported to induce their expression at the mRNA or protein level. However, where these transporters increase in liver was not clear. Previously we showed that feeding rats an HF/HS diet containing 0.5% cholesterol and 0.25% cholic acid produced severe hepatic steatosis. Thus we investigated whether diet-induced Abcg5 and Abcg8 expression occurs in bile canaliculi using this diet. First, we compared the expression levels of Abcg5/Abcg8 proteins in the bile canaliculi-rich fraction prepared from mice fed the HF/HS diet with those in the fraction from mice fed the SD diet. Consistent with our previous finding in rats, the HF/HS diet given for 3 weeks caused severe hepatic steatosis (Table 1, Supplemental Fig. 1). In the bile canaliculi-rich fraction prepared from mice fed the HF/HS diet, levels of Abcg5 and Abcg8 were notably elevated, while no significant differences in other hepatic transporters were detected (Fig. 2). Next, using immunohistochemistry, we investigated whether the enhanced expression of Abcg5/Abcg8 is restricted to bile canaliculi. In accordance with the immunoblot data, immunohistochemistry showed marked increases of Abcg5 and Abcg8 only in bile canaliculi from cryosections of the HF/HS diet-fed mice (Fig. 3). These results suggest that the HF/HS diet induced marked cholesterol accumulation in liver, which led to increases in the expression of Abcg5/Abcg8 proteins in bile canaliculi so as to secrete the excess cholesterol into bile.

The regulation of Abcg5/Abcg8 expression is thought mostly to occur at the transcriptional level via orphan nuclear receptors. The liver X receptor (LXR/Lxr) is a well-known receptor which contributes to the protein expression of Abcg5/Abcg8. However, its involvement in diet-induced Abcg5/Abcg8 protein expression in bile canaliculi remains to be elucidated. Further, whether the...
protein expression of Abcg5/Abcg8 by LXR/Lxr agonists actually occurs in bile canaliculi has not been verified. Consequently, we analyzed the bile canaliculi-rich fraction and liver sections from mice administered TO901317. However, the increases in the levels of Abcg5 and Abcg8 caused by the LXR/Lxr agonist were much lower than those due to the HF/HS diet (Figs. 2, 4 and Table 2). Further feeding of the HF/HS diet failed to induce the gene expression of Lxrα, or other transcription factors which up-regulate Abcg5/Abcg8 expression, such as Fxr and FoxO1, in mouse liver. As far as we know, in some animal models a high-fat diet increases the hepatic gene expression of Lxrα, and in others it fails to do so.37-40 These observations suggest post-translational regulation rather than transcriptional regulation to be important in this event.

The diet-induced increase in the protein levels of hepatic transporters appears to be caused by post-translational regulation. Muller demonstrated that a significant 2.5-fold increase in the protein level of Abcb4 occurred in response to a lithogenic diet without a change in the mRNA level.41 However, Kok showed that treatment with a peroxisome proliferator-activated receptor alpha (PPARα) agonist resulted in a 2.5-fold increase in the protein level of Abcb4 and a 3-fold increase in the mRNA level.42 These results are similar to the diet- or LXR/Lxr agonist-induced expression of Abcg5/Abcg8 in our study. Furthermore, Kipp demonstrated that the half-lives of ABC transporters, e.g., rat Abcb1 (Mdr1), Abcb4, and Abcb11, are 4–6 days, and most of these transporters are recruited from the intracellular pool corresponding to a recycling endosome in hepatocytes when needed.43,44 Considering our observations, it appears that Abcg5 and Abcg8 are recruited from such pools in hepatocytes so as to secrete the excess cholesterol into bile. However, most studies have focused on transcriptional regulation, but the intracellular trafficking of Abcg5 or Abcg8 remains to be clarified. To elucidate the mechanisms behind the regulation of these transporters, an analysis of their behavior after translation will be needed.

In conclusion, we demonstrated that in mice, consumption of an HF/HS diet caused excessive loading of the liver with cholesterol, and induced the expression of the Abcg5/Abcg8 proteins in bile canaliculi. Administration of TO901317, a LXR/Lxr agonist, also up-regulated the expression of Abcg5/Abcg8 in bile canaliculi; however the increases in the protein levels of Abcg5/Abcg8 in bile canaliculi in the HF/HS diet-fed mice were much higher than those in TO901317-administered mice despite similar increases in the mRNA levels in both mice. Together with the down-regulation of the gene expression of Lxr and other transcription factors in the HF/HS diet-fed mice, post-translational regulation rather than transcriptional regulation may be important. To clarify the mechanism of Abcg5/Abcg8 expression in bile canaliculi, a method of monitoring the intracellular trafficking of these proteins in liver is now under development.

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