Arginine methylation is responsible for diverse biological functions and is mediated by protein arginine methyltransferases (PRMTs). Nonalcoholic fatty liver disease (NAFLD) is accompanied by excessive hepatic lipogenesis via liver X receptor α (LXRα). Thus we examined the pathophysiological role of PRMTs in NAFLD and their relationship with LXRα. In this study, palmitic acid (PA) treatment increased PRMT3, which is correlated with the elevation of hepatic lipogenic proteins. The expression of lipogenic proteins was increased by PRMT3 overexpression, but decreased by PRMT3 silencing and use of the PRMT3 knockout (KO) mouse embryonic fibroblast cell line. PRMT3 also increased the transcriptional activity of LXRα by directly binding with LXRα in a methylation-independent manner. In addition, PA treatment translocated PRMT3 to the nucleus. In animal models, a high-fat diet increased the LXRα and PRMT3 expressions and binding, which was not observed in LXRα KO mice. Furthermore, increased PRMT3 expression and its binding with LXRα were observed in NAFLD patients. Taken together, LXRα and PRMT3 expression was increased in cellular and mouse models of NAFLD and human patients, and PRMT3 translocated into the nucleus bound with LXRα as a transcriptional cofactor, which induced lipogenesis. In conclusion, PRMT3 translocation by PA is coupled to the binding of LXRα, which is responsible for the onset of fatty liver.

Nonalcoholic fatty liver disease (NAFLD) is a worldwide metabolic syndrome defined by an increased accumulation of fat, mainly triglycerides (TGs), within hepatocytes. NAFLD is closely related to diabetes because its pathogenesis is accompanied by hepatic insulin resistance and excessive hepatic lipogenesis (1,2). Many studies have been conducted to verify the molecular mechanism of lipogenesis. However, the whole mechanism is highly complex and difficult to understand.

In the liver, liver X receptor α (LXRα) regulates SREBP1c and carbohydrate-responsive element-binding protein (ChREBP), which induce lipogenesis (3,4). LXRα that has been activated by oxysterol, an endogenous ligand, and/or T0901317, an exogenous ligand, increases the expression and transcriptional activity of SREBP1c and ChREBP and subsequently increases the transcription of fatty acid synthase (FAS) and acetyl CoA carboxylase (ACC), which are lipogenic enzymes (5–7). The transcriptional activity of LXRα is regulated by its interaction with its transcriptional cofactor, which determines whether binding with LXRα response element (LXRE) will occur (8,9). LXRα-deficient mice have shown markedly lower hepatic expression of SREBP1c and its target genes (10). Moreover, T0901317-induced hepatic expression of ACC and FAS was attenuated in ChREBP-knockout (KO) mice (4). Many studies have demonstrated the obvious role of LXRα in lipogenesis through SREBP1c and/or ChREBP, but recent studies...
also suggested the dispensable role of LXRα activation for the SREBP1c- and ChREBP-dependent lipogenesis (11,12).

Protein arginine methyltransferases (PRMTs) are thought to be new biomarkers that regulate epigenetic events and posttranslational modification (13). PRMTs methylate histone and nonhistone proteins in arginine residue. The nine mammalian forms of PRMTs can be divided into three subtypes. Type I (PRMTs 1, 2, 3, 4, 6, and 8) enzymes catalyze arginine residue to a monomethylarginine intermediate, which is then converted to asymmetric dimethylarginine (ADMA).

In the past two decades, many studies have reported that increased ADMA production associated with nitric oxide synthase was related to the onset of heart failure and/or chronic kidney disease (14,15). However, because protein methylation is one of the most abundant protein modifications and ADMA is catalyzed by type I PRMTs, current studies have focused on arginine methylation as a molecular signal implicated in various pathophysiological diseases, including cancer and metabolic syndrome. Hong et al. (16) recently reported the expression of PRMTs in male rat tissues; they found that PRMT1, PRMT4, and PRMT5 were minimally expressed in the liver. However, several lines of evidence have shown the importance of type I PRMTs in the liver, as they are responsible for hepatic glucose metabolism. Choi et al. (17) and Han et al. (18) reported that PRMT1 and PRMT6 regulate hepatic gluconeogenesis. Lee et al. (19) also demonstrated an increased ADMA plasma concentration and decreased hepatic PRMT1 expression in diabetic mice (db/db), suggesting that PRMTs play an important role in the onset of diabetes in the liver. Kroenes-Herzig et al. (20) suggested that PRMT4 is a critical component of cyclic adenosine monophosphate–dependent glucose metabolism in the liver.

Another important isoform of type I PRMT is PRMT3, which induces ADMA methylation. Its substrates are very restricted because of its predominant cytoplasmic location (21). The role of PRMT3 in dendritic spine maturation was recently reported. However, the functional role of PRMT3 in metabolic disorders has not been elucidated. Thus we examined the role of PRMT3 in hepatic lipogenesis using a hepatocyte cell line, a high-fat diet animal model, and NAFLD patients.

**RESEARCH DESIGN AND METHODS**

**Reporter Gene Assay**

Cells were transiently transfected with indicated plasmids in addition to β-galactosidase and luciferase plasmids. After 24 h, the culture medium was changed with medium containing indicated concentrations of drugs and incubated for an additional 6 h. The cells were lysed with passive lysis buffer (E194A; Promega), and then cell extracts were analyzed. Luminescence was analyzed with GloMax Microplate Reader. Final 1 mmol/L luciferin (E1602; Promega) was dissolved in luciferase assay buffer (1 mol/L KH₂PO₄, pH 7.8, 1 mol/L MgCl₂, and 0.1 mol/L ATP). β-galactosidase plasmid was used as a control for the normalization of transfection efficiency.

**Chromatin Immunoprecipitation**

Chromatin immunoprecipitation assay was performed using the EZ-ChIP kit (Millipore, Billerica, MA) as instructed by the manufacturer. Briefly, cultured cells were fixed with 1% formaldehyde and harvested. Cell pellets were lysed in SDS lysis buffer, and sonication was performed to share chromatin. Shared chromatin was precleared with protein G agarose, then normal mouse IgG/LXRα antibody or normal rabbit IgG/PRMT3 antibody was added. After 17 h, immune complex was incubated with protein G agarose for 2 h. Precipitated chromatin were eluted, then protein/DNA crosslinking was reversed. Purified DNA was amplified by real-time quantitative PCR using the following primers; forward 5’–GCCAGGACTTCTCTGTTGTA-3’ and reverse 5’–CTGATGGTTGTTGGTTACT-3’ for -461/-326 region of human SREBP1c promoter.

**Human Liver Tissue**

Surgical liver resections were performed from 10 patients (four men, six women; mean age, 60.4 years; range, 49–74 years). Control nonfatty liver samples were collected from five patients undergoing hepatic resection for intrahepatic bile duct stone (n = 5). Fatty liver samples were collected from fatty livers of five patients. All materials were used under the protocol approved by the Institutional Review Board at Chonnam National University Hospital.

**Proximity Ligation Assay**

Paraffin sections were deparaffinized with HistoChoice (Amresco) and rehydrated with serial diluted ethanol. Sections were steamed in sodium citrate buffer (10 mmol/L sodium citrate, 0.05% Tween 20, pH 6.0) for antigen retrieval and washed with PBS. Then proximity ligation assay (PLA) was performed using Duolink (Olink Biosciences, Uppsala, Sweden) according to the manufacturer’s instructions. Briefly, the sections were incubated with primary antibodies (anti-LXRα and anti-PRMT3) diluted to 1:100 in antibody diluents supplied for overnight at 4°C. The Duolink PLA probes were incubated in preheated humidity chamber for 1 h at 37°C, followed by ligation, amplification. To reduce tissue autofluorescence, sections were incubated with copper sulfate (5 mmol/L CuSO₄ in 50 mmol/L ammonium acetate buffer, pH 5.0) for 20 min (22), followed by mounting. The stained sections were observed using Leica TCS SP5 AOB5 laser scanning confocal microscope (Leica Microsystems, Heidelberg, Germany) using a Leica 63× (numerical aperture 1.4) oil objective located at Korea Basic Science Institute Gwangju Center.

**Statistical Analysis**

The results were expressed as mean ± SEM. Values are mean ± SEM of three or four independent experiments. For two group comparisons, a Student t test was used, and for multiple comparisons, one-way ANOVA by SPSS (SPSS Inc., Chicago, IL), followed by the Tukey post hoc test, was used. A value of P < 0.05 was considered significant.
RESULTS
Palmitic Acid Treatment Alters Arginine Asymmetric Dimethylation Status and Increases PRMT3 Expression and Hepatic Lipogenesis

Although the importance of protein arginine asymmetric dimethylation in various tissues is increasingly recognized, the functional role of hepatic arginine asymmetric dimethylation in hepatic lipogenesis is poorly understood. To confirm the changes in ADMA levels induced by saturated fatty acids, HepG2 cells were treated with 750 μmol/L palmitic acid (PA), followed by ASYM24 antibody, which recognizes ADMA. Twenty-four hours after this PA treatment, many dynamic changes in protein arginine asymmetric dimethylation were observed. Pretreatment with adenosine-2',3'-dialdehyde (Adox), a global methylation inhibitor, blocked the arginine asymmetric dimethylation status in the control and PA groups (Fig. 1A). This result suggests that type I PRMTs are associated with hepatocyte function, as they induce arginine asymmetric dimethylation. Thus we examined the effect of PA on type I PRMT expression. PA treatment significantly increased PRMT3 expression (Fig. 1B), and PA only slightly increased PRMT1 expression and did not alter PRMT4 expression. PRMT1 was recently reported to have a functional role in hepatic gluconeogenesis (17). Based on this finding, we examined the role of PRMT3 in hepatic lipogenesis. PA

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**Figure 1**—PA changes the arginine asymmetric dimethylation status and increases PRMT3 expression and hepatic lipogenesis. A: After pretreatment with 30 nmol/L Adox for 30 min, HepG2 cells were treated with 750 μmol/L PA for 24 h. Cell extracts were subjected to Western blot analysis with indicated antibodies and then normalized to the β-actin level. Data are mean ± SEM of three independent experiments. *P < 0.05 vs. control; †P < 0.05 vs. PA treatment. B: HepG2 cells were treated with 750 μmol/L PA for 12 and 24 h. Type I PRMTs were assayed by Western blot analysis with the indicated antibodies and then normalized to the β-actin level. Data are mean ± SEM of five independent experiments. *P < 0.05 vs. 0 h. C: HepG2 cells were treated with 750 μmol/L PA for the indicated time intervals. Cell extracts were subjected to Western blot analysis with the indicated antibodies and then normalized to the β-actin level. Data are mean ± SEM of five independent experiments. *P < 0.05 vs. 0 h. D: HepG2 cells were treated with 750 μmol/L PA for 24 h. D: Cultured cells were subjected to Oil Red O staining then counterstained with hematoxylin. Representative images were from at least three independent experiments. E: Cell extracts were subjected to Western blot analysis with the indicated antibodies and then normalized to the β-actin level. Data are mean ± SEM of five independent experiments. *P < 0.05 vs. control; †P < 0.05 vs. PA.
increased the level of PRMT3 mRNA (Supplementary Fig. 1A), which is correlated with the levels of PRMT3 proteins. We also examined the time-dependent effect of PA on the expression of PRMT3, LXRα, and other lipogenic proteins. PA treatment increased PRMT3, LXRα, and other lipogenic proteins. PA treatment increased PRMT3, LXRα, cleaved SREBP1c (cl-SREBP1c; which is an active form of SREBP1c [23]), FAS, and total ACC expression, but not peroxisome proliferator-activated receptor γ, ChREBP expression, and ACC phosphorylation, in a time-dependent manner (Fig. 1C and Supplementary Fig. 1B). These results suggest that the protein arginine methylation status is important in hepatic lipogenesis. Indeed, PA-induced lipid accumulation and expression of lipogenic proteins were blocked by treatment with Adox (Fig. 1D and E). However, ChREBP expression was not influenced by PA and Adox treatment (Supplementary Fig. 1C).

**PRMT3 Regulates Hepatic Lipogenesis Parameters Through LXRα Signaling**

To reveal the functional role of PRMT3, transient transfection of PRMT3 was conducted in HEK293 cells. Overexpression of hemagglutinin (HA)-tagged PRMT3 increased the expression of lipogenic proteins, including LXRα, FAS, ACC, and cl-SREBP1c but did not alter the expression of peroxisome proliferator-activated receptor γ, ChREBP, p-ACC, and PEPCK (Fig. 2A and Supplementary Fig. 2A). These results suggest that PRMT3 increases lipogenic protein expression. We examined this effect further using PRMT3 KO mouse embryonic fibroblast (MEF) cells. We found markedly decreased expression of LXRα, cl-SREBP1c, FAS, and total ACC in KO cells compared with wild-type (WT) cells (Supplementary Fig. 2B). Moreover, the PA-induced upregulation of lipogenic proteins observed in the WT MEF cells did not occur in the PRMT3 KO cells (Fig. 2B). Indeed, PRMT3 silencing by small interfering RNA (siRNA) transfection blocked PA-induced lipogenic proteins, including LXRα, cl-SREBP1c, FAS, and total ACC, in HepG2 cells (Fig. 2C). However, ChREBP and PEPCK expressions were not influenced by PRMT3 expression as well (Supplementary Fig. 2C and D). In addition, PA-induced lipid accumulation, fatty acid synthesis, and TG accumulation were blocked by PRMT3 silencing in HepG2 cells (Fig. 2D–F and Supplementary Fig. 2E). LXRα promotes cholesterol efflux by increase of ATP-binding cassette (ABC) transporter isoforms, those are ABCA1, ABCG1, ABCG5, and ABCG8 (24,25). However, PRMT3 silencing did not alter the expression of these genes and intracellular cholesterol level (Supplementary Fig. 2F–H). These results suggest that PRMT3 regulates LXRα SREBP1c-dependent hepatic lipogenesis. To further confirm this signaling axis, HA or HA-PRMT3 plasmid was transfected to AML-12 cells, normal mouse hepatocyte, with scramble or LXRα siRNA. PRMT3-induced expression of lipogenic proteins and lipid accumulation were abolished by LXRα silencing (Fig. 2G and Supplementary Fig. 2I and J). These results suggest that LXRα is required for PRMT3-induced expression of lipogenic proteins.

**PRMT3 Upregulates the Transcriptional Activity of LXRα**

To evaluate whether overexpression of PRMT3 increases the transcriptional activity of LXRα, we performed a reporter gene assay using LXRE or native SREBP1c promoter (up to 0.9 Kb) containing luciferase plasmids in HepG2 cells. Treatment with T0901317, an exogenous LXRα ligand, increased the transcriptional activity of LXRα (Fig. 3A and C). In addition, overexpression of PRMT3 increased the transcriptional activity of LXRα. Treatment with T0901317 significantly increased the activity of LXRα in PRMT3-overexpressed cells compared with green fluorescent protein (GFP)-overexpressed cells. Moreover, PA treatment further increased the transcriptional activity of LXRα. Interestingly, Adox treatment did not influence the transcriptional activity of LXRα in PRMT3-overexpressed cells (Fig. 3A and C). Similar result was observed in HEK293 cells (Supplementary Fig. 3A). These results suggest that PRMT3 overexpression increases LXRα activity, but not via PRMT3-mediated methylation. For further confirmation, we conducted PRMT3 siRNA transfection in HepG2 cells. As expected, silencing of PRMT3 attenuated the T0901317- and PA-induced transcriptional activity of LXRα (Fig. 3B and D). These results suggest that PRMT3 is an intermediate molecule to represent the effect of LXRα in hepatocytes.

To evaluate that PRMT3 could directly regulate transcriptional activity of SREBP1c or ChREBP, HepG2 cells were transfected with SREBP response element or carbohydrate response element luciferase plasmids. However, PRMT3 overexpression altered neither of their transcriptional activities (Supplementary Fig. 3B and C).

**PRMT3 Binds with LXRα In Vitro and in Intact Cells, Then Acts as a Transcriptional Cofactor**

To verify that increased transcriptional activity of LXRα is involved in direct binding of PRMT3, we conducted a glutathione s-transferase (GST) pull-down assay. Overexpressed GST and GST-PRMT3 proteins in the BL21 strain of Escherichia coli were purified with glutathione sepharose beads. The purified proteins were then incubated with HEK293 whole cell lysate, which is overexpressed with HA-tagged LXRα. LXRα strongly interacted with GST-PRMT3, but not with GST alone (Fig. 4A). To elucidate whether the interaction between PRMT3 and LXRα also occurs in intact cells, we cotransfected Flag-PRMT3 with HA or HA-LXRα in HEK293 cells. The whole cell lysate was then immunoprecipitated with M2 antibody, which specifically recognizes Flag. Immunoprecipitation was reciprocally conducted with HA antibody. As in the GST pull-down assay, we confirmed that the interaction between PRMT3 and LXRα also occurred in intact cells (Fig. 4B).

PRMT3 is an enzyme that can methylate the arginine residue of a substrate. To verify whether direct binding between LXRα and PRMT3 occurs because of LXRα methylation, we performed an in vitro methylation assay. Purified GST-PRMT3 was incubated with HA–ribosomal
protein S2 (rpS2) or HA-PRMT3 extract purified from an overexpressed HEK293 cell lysate, and S-adenosyl-L-[methyl-3H]methionine (methyl donor) was then added. Purified GST-PRMT3 methylated rpS2, a PRMT3 substrate, but not LXRα (Fig. 4C). To further confirm, we conducted chromatin immunoprecipitation with LXRα and PRMT3 antibodies. PA treatment recruited LXRXα and PRMT3 to the LXREs of SREBP1c promoter region (Fig. 4D). However, PA-induced recruitment of LXRXα was abolished by PRMT3 silencing (Fig. 4D). Thus we suggest that PRMT3 directly binds to LXRXα, then acts as a transcriptional cofactor without LXRXα methylation.

**PA Treatment Induces the Translocation of PRMT3 to the Nucleus**

PRMT3 location is reportedly restricted to the cytosol fraction in some cell lines (21). PRMT3 might bind with LXRXα in the nucleus because LXRXα is a nuclear receptor.
To identify the colocalization of PRMT3 and LXRα in the nucleus, we cotransfected GFP-PRMT3 and HA-LXRα in HEK293 cells, then conducted immunofluorescence with anti-tetramethylrhodamine-5-(and 6)-isothiocyanate antibody for HA staining. Stained cells were observed with confocal microscopy. Overexpressed GFP-PRMT3 was located in the cytoplasm (Fig. 5A). However, PA treatment increased the nuclear location of PRMT3 and colocalization with LXRα, despite the presence of some cytosolic PRMT3 (data not shown). The experiment using an HA-tagged PRMT3 construct stained with anti-fluorescein isothiocyanate antibody also validated the fact that the nuclear expression of PRMT3 was increased by PA treatment in HEK293 cells (Supplementary Fig. 4A). These results suggest that in spite of the dominant location of PRMT3 in the cytosol, PA treatment can increase the translocation of exogenous PRMT3 to the nucleus.

To investigate whether nuclear translocation of endogenous hepatic PRMT3 occurs in response to PA, we conducted immunofluorescence staining for the endogenous PRMT3 in HepG2 cells. Endogenous PRMT3 was stained with anti-fluorescein isothiocyanate antibody. When treated with vehicle, PRMT3 was located in the...
cytoplasm and nucleus (Fig. 5B and Supplementary Fig. 4B). However, PA treatment increased the nuclear accumulation of PRMT3. Interestingly, T0901317 treatment recruited almost all cytosolic PRMT3 to the nucleus. These results show that endogenous hepatic PRMT3 is located not only in the cytoplasm, but also in the nucleus, and that PA and the exogenous LXRα ligand increase the nuclear accumulation of PRMT3 in HepG2 cells.

To further confirm these results, HepG2 cells were treated with PA, and the nuclear fraction was then extracted. Similar to the immunofluorescence results, PA treatment simultaneously increased nuclear PRMT3, LXRα, and nuclear SREBP1c expression (Fig. 5C).

Expression and Binding of LXRα and PRMT3 Are Increased in the Livers of Mice Fed a High-Fat Diet

To validate that this event also takes place in mouse models, we fed mice a 60% high-fat diet for 12 weeks. As expected, high-fat-fed mice exhibited increased hepatic PRMT3 and LXRα expression, as well as lipogenic protein expression, compared with mice on a normal chow diet (Fig. 6A). Furthermore, PRMT3 and LXRα expression increased in the nuclear fraction isolated from the livers of high-fat-fed mice (Fig. 6B). To examine whether binding between LXRα and PRMT3 manifests in mouse models, we conducted immunoprecipitation with PRMT3 antibody from mouse liver lysates. Increased binding between

Figure 4—PRMT3 binds with LXRα and acts as a transcriptional cofactor. A: HEK293 cells were transfected with HA-LXRα plasmid. Twenty-four hours later, total proteins were extracted and incubated with purified GST/GST-PRMT3–fused glutathione sepharose beads overnight. In vitro binding was assessed with HA antibody. Purified GST/GST-PRMT3 proteins were confirmed by Coomassie blue staining, and transfection efficacy was assessed with HA antibody from HEK293 lysate. B: HEK293 cells were cotransfected with Flag-PRMT3 and HA/LXRα plasmids. Twenty-four hours later, total proteins were extracted, and reciprocal coimmunoprecipitation was conducted with Flag or HA antibodies and immunoblotted with the indicated antibodies. Expression of Flag, HA, and PRMT3 from 5% input were analyzed by immunoblotting. C: HEK293 cells were transfected with HA-rpS2 or HA-LXRα plasmids. Twenty-four hours later, total proteins were extracted and in vitro methylation assay was performed following the method introduced in RESEARCH DESIGN AND METHODS. D: HepG2 cells were transfected with scramble siRNA or PRMT3 siRNA following the reverse transfection method. Twenty-four hours later, the cells were treated with 750 μmol/L PA. Eight hours later, the cells were treated with final 1% of formaldehyde then chromatin immunoprecipitation assay was performed with LXRα or PRMT3 antibodies following the method introduced in RESEARCH DESIGN AND METHODS. *P < 0.05 vs. scramble; †P < 0.05 vs. scramble + PA. IP, immunoprecipitation; WB, western blotting. (A high-quality color representation of this figure is available in the online issue.)
LXRα and PRMT3 was observed in high-fat-fed mice compared with mice on a normal chow diet (Fig. 6C). However, this binding was not observed in high-fat-fed LXRα-deficiency mice (Fig. 6C); and LXRα deficiency did not influence high-fat diet–induced PRMT3 expression (Fig. 6C; input). These results suggest that a high-fat diet increases lipogenesis via increased PRMT3 and LXRα expression and binding in mouse models.

**PRMT3 Expression and Binding of PRMT3 and LXRα Are Increased in the Livers of NAFLD Patients**

To confirm that increased PRMT3 expression and binding of PRMT3 and LXRα are observed in human liver, we used the liver tissues from nonfatty liver patients and NAFLD patients. As expected, dramatically increased PRMT3 expression was observed in liver from NAFLD patients compared with liver from nonfatty liver patients (Fig. 7A). In addition, lots of PRMT3-positive cells were stained in the nucleus (Fig. 7A; red arrow). Binding between PRMT3 and LXRα, determined by PLA, was also increased in NAFLD patients (Fig. 7B). These results suggest that increased hepatic PRMT3 expression and binding with LXRα promote the progression of NAFLD in humans.

**DISCUSSION**

NAFLD, an outstanding example of the complex pathophysiology of metabolic syndrome, is considered to be

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**Figure 5**—PA treatment induces the translocation of PRMT3 to the nucleus. A: HEK293 cells were cotransfected with GFP-PRMT3 and HA-LXRα plasmids. Twenty-four hours later, the cells were treated with 750 μmol/L PA for 12 h then fixed, immunostained with HA antibody, and evaluated. Green, GFP-PRMT3; red, HA-LXRα; blue, DAPI. B: HepG2 cells were treated with 750 μmol/L PA or 10 μmol/L T0901317 for 12 h then fixed, immunostained with PRMT3 antibody, and evaluated. Green, endogenous PRMT3; blue, DAPI. C: HepG2 cells were treated with 750 μmol/L PA for 12 and 24 h. Nuclear proteins were extracted then subjected to Western blot analysis with the indicated antibodies and finally normalized to the lamin B level. Data are mean ± SEM of three independent experiments. *P < 0.05 vs. 0 h. DIC, differential interference contrast; Pal, palmitic acid.

**Figure 6**—Expression and binding of LXRα and PRMT3 are increased in the livers of mice fed a high-fat diet. A: C57BL/6J mice were challenged with normal or high-fat diet for 12 weeks. Total proteins were extracted from the liver, and tissue extracts were subjected to Western blot analysis with the indicated antibodies. Representative immunoblots were from at least three independent experiments. B: Nuclear proteins were extracted from the liver, subjected to Western blot analysis with the indicated antibodies, and then normalized to the lamin B level. Data are mean ± SEM of three independent experiments. *P < 0.05 vs. normal diet. C: LXRα WT and KO mice were challenged with a normal or high-fat diet for 12 weeks. Endogenous PRMT3 in the tissue extracts was immunoprecipitated with PRMT3 antibody and immunoblotted with LXRα antibody. Expression of PRMT3 and β-actin from 5% input were analyzed by immunoblotting. Representative immunoblots were from at least three independent experiments. HFD, high-fat diet; IP, immunoprecipitation; ND, normal diet; WB, western blotting.
among the most common liver diseases worldwide. However, to our knowledge, hepatic ADMA status and type I PRMTs in hepatic lipid metabolism have not yet been assessed. In this study, we first showed that PA treatment alters ADMA status and type I PRMTs in hepatocytes. Interestingly, Adox treatment markedly reduced the PA-induced ADMA status and lipogenesis, including lipid accumulation and related lipogenic molecules, indicating that type I PRMTs usually play important roles in hepatic lipid metabolism. Our study revealed that PA also slightly increased the expression of PRMT1, which is consistent with a previous report on hepatic glucose metabolism (17). However, in this study, we focused on the as-yet unidentified role of PRMT3 in lipid metabolism in hepatocytes because PA markedly increased the expression of PRMT3 in type I PRMTs. Based on these results, we hypothesized that asymmetric dimethylation by PRMT3 would be involved in high-fat-induced hepatic lipogenesis. This hypothesis was supported by several findings: 1) PA activated lipogenesis and PRMT3 expression in hepatocytes, 2) overexpression of HA-PRMT3 induced lipogenesis, 3) PA-induced lipogenesis was not observed in the PRMT3 KO MEF cell line, and 4) PRMT3 siRNA reduced PA-induced lipid accumulation in hepatocytes.

LXRs are known regulators of fatty acid metabolism in various cells, including hepatocytes, adipocytes, and pancreatic β-cells (26–28). Recent studies have revealed that high-fat diets increase the expression of lipogenic proteins via LXRα in the liver (26,29,30). In the current study, we also confirmed that PA treatment increases not only LXRα expression, but also lipid accumulation in HepG2 cells, which is consistent with the finding of Kuang et al. (31) that PA stimulates LXR activities. LXRα is considered to be a master of lipogenesis in the liver because of its functional role of SREBP1c and

Figure 7—PRMT3 expression and binding of PRMT3 and LXRα are increased in the livers of NAFLD patients. A and B: Human liver tissues from nonfatty liver patients and NAFLD patients were prepared as introduced in RESEARCH DESIGN AND METHODS. A: Immunohistochemistry using paraffin sections was performed with PRMT3 antibody. Representative images were from at least three independent experiments. (yellow arrows, PRMT3-positive cells; red arrows, PRMT3-positive cells stained in nucleus) B: PLA using paraffin sections was performed with anti-rabbit PRMT3 antibody and anti-mouse LXRα antibody (green fluorescence, binding of PRMT3 and LXRα). Representative images were from at least three independent experiments. C: Summarized scheme. DIC, differential interference contrast; HFD, high-fat diet.
ChREBP regulation (32). However, the current study revealed that PRMT3 regulates only the expression of lipid metabolism molecules, such as LXRα and SREBP1c, but not ChREBP or PEPCK. This result is supported by a report showing that glucose is required for ChREBP functional activity and that LXRs are not necessary for the induction of glucose-regulated genes in the liver, suggesting that ChREBP and LXRs are independent (12). LXRx also regulates cholesterol efflux through its receptor expression (25). However, in this study, expression of ABC transporter isomorphs and intracellular cholesterol level were not altered by PRMT3 silencing. These results suggest that effect of PRMT3 on LXRx activity has very selective targets such as SREBP1c but not ChREBP or ABC transporters. Until now, no report has shown that PRMT3 is an upstream regulator of LXRx activation in the liver. The current study showed that PRMT3 overexpression increases SREBP1c native promoter activity and that silencing of PRMT3 decreases the binding of LXRα to the SREBP1c promoter region. Based on these results, we suggest that PA treatment induces hepatic lipogenesis via the PRMT3-LXRx-SREBP1c axis.

Striking observations in this study were that PRMT3 bound with LXRx in vitro and in vivo and increased LXRE-dependent transcriptional activity. However, Adox treatment did not prevent the transcriptional activity of LXRx. Moreover, LXRx was not methylated by PRMT3. These results suggest that the PRMT3-induced transcriptional activity of LXRx is methyltransferase independent. Although LXRx has a few RxR motifs (arginine–x amino acid–arginine) that often act as the methylation sites of PRMT substrates (33,34), PRMT1 and PRMT4 did not methylate LXRx in the current study (data not shown), suggesting that type I PRMTs do not directly methylate LXRx. Furthermore, we observed that PRMT3 binds with LXRx in the livers of mice on a high-fat diet and NAFLD patients. Based on our results, we speculate that methylation is an important modification of hepatic lipogenesis. However, upregulation of LXRx transcriptional activity by PRMT3 is not associated with the enzymatic activity of PRMT3. LXRx activation induced by T0901317 has been known to increase the transcription of its target genes via trimethylation of H3K4 (35,36). In this study, as PA-induced lipogenesis was blocked by Adox, it may be speculated that reduced trimethylation of H3K4 by Adox is linked to the diminished lipogenesis. Further studies should be performed to reveal this speculation.

PRMT3 has limited substrates because its location is restricted (21). rpS2, which balances between ribosomal subunits, is a well-known substrate of PRMT3. Most studies of PRMT3 have revealed that it is located exclusively within the cytoplasm (37–39). Consistent with these reports, we observed an overwhelming cytoplasmic portion of GFP-PRMT3. However, we observed that PA treatment caused some translocation of PRMT3 to the nucleus, while a large portion remained in the cytoplasm. Like GFP, HA-PRMT3 was observed in the nucleus after PA treatment, ruling out the possible problem of GFP tagging. Moreover, we found that endogenous PRMT3 was recruited to the nucleus by treatment of HepG2 cells with T0901317. These results suggest that the coupling of PRMT3 and LXRx is very important for lipogenesis in the liver. To our knowledge, evidence of the location of PRMT3 in the nucleus is restricted to a report by Tavanez et al. (40), which stated that PRMT3 accumulates in intranuclear inclusions of muscle in patients with oculopharyngeal muscular dystrophy. These authors did not evaluate the translocation of PRMT3. The current study provides novel evidence that PRMT3, which is known to be restricted to the cytosolic fraction, translocates to the nucleus in the hyperlipidemic condition. Furthermore, the result of chromatin immunoprecipitation assay, performed with PRMT3 antibody, is another strong evidence that PRMT3 exists in the nucleus. PRMT proteins were found to be implicated in epigenetic events by methylating arginine residue of histone tail (41). However, histone arginine methylation by PRMT3 has not been discovered yet. A recent study reported that LXRx target gene expression is regulated by histone modification (36). Our present findings along with previous reports may suggest that LXRx targets genes selectively being introduced by nuclear PRMT3-mediated histone modification. Further studies on the possible mechanisms by which PRMT3 selectively increases LXRx target genes by modifying histone will bring new insight into the roles of PRMT3.

LXRx agonists, such as T0901317 and GW3965, have been considered to be potent therapeutic agents against atherosclerosis, obesity, and even cancer (42,43). However, because of their side effects, including increased serum TG levels secondary to hepatic lipogenesis (44), their use has been limited. Many studies have been conducted to determine how to overcome these side effects (45). In this study, we found that PRMT3 regulates LXRx activity. We suggest that the targeting of PRMT3 could be a new therapeutic approach to reduce the side effects of LXRx.

In conclusion, as described in Fig. 7C, we showed that 1) PA treatment increased PRMT3 expression and hepatic lipogenesis, 2) PRMT3 increased LXRx activity and its target gene expression by direct binding in a methylation-independent manner, 3) PA treatment increased the translocation of PRMT3 from the cytosol to the nucleus, and 4) PRMT3 and LXRx expression and binding increased in high-fat-fed mice and NAFLD patients. Our findings provide novel evidence that PRMT3 regulates hepatic lipogenesis via interaction with LXRx. Targeting of PRMT3 is a potential approach to the treatment of NAFLD and prevention of the side effects of the LXRx agonist.

Acknowledgments. The authors thank Mark T. Bedford (The University of Texas MD Anderson Cancer Center, Smithville, TX) for permitting to use PRMT constructs and PRMT3 KO MEF cell lines. HA-PRMT constructs were kindly provided by Akiyoshi Fukamizu (Life Science Center of Tsukuba Advanced Research Alliance, University of Tsukuba, Japan).
Funding. This work was supported financially by a basic science research grant from the National Research Foundation of Korea (2010-0023627). The animal experiment in this study was partially supported by the Animal Medical Institute of Chonnam National University.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. D.-i.K. contributed to experimental design, performed the experiments, researched data, wrote and reviewed the manuscript, and contributed to the revision of the manuscript. M.-j.P. performed the animal experiments, researched data, and contributed to discussion and revision of the manuscript. S.-L.K. researched data and contributed to discussion. J.-i.P. performed the confocal microscope experiments and contributed to discussion. K.-c.Y., H.-j.H., and J.-A.G. researched data and contributed to the critical review and revision of the manuscript. J.-h.L. contributed to experimental design and contributed to the critical review and revision of the manuscript. S.-h.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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