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Oxysterol Sulfation by SULT2B1b Suppresses LXR/SREBP-1c Signaling Pathway and Reduces Serum and Hepatic Lipids in Mouse Models of NAFLD

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Abstract

Background—Cytosolic sulfotransferase (SULT2B1b) catalyzes oxysterol sulfation. 5-cholesten-3β-25-diol-3-sulfate (25HC3S), one product of this reaction, decreases intracellular lipids *in vitro* by suppressing LXR/SREBP-1c signaling, with regulatory properties opposite to those of its precursor 25-hydroxycholesterol (25HC). Up-regulation of SULT2B1b may be an effective strategy to treat hyperlipidemia and hepatic steatosis.

Objectives—To explore the effect and mechanism of oxysterol sulfation by SULT2B1b on lipid metabolism *in vivo*.

Methods—C57BL/6 and LDLR$^{-/-}$ mice were fed with high-cholesterol diet (HCD) or high-fat diet (HFD) for 10 weeks, and infected with adenovirus encoding SULT2B1b. SULT2B1b expressions in different tissues were determined by immunohistochemistry and western blot. Sulfated oxysterols in liver were analyzed by HPLC. Serum and hepatic lipid levels were

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Conflict of interest
The authors declare that they have no conflict of interest.

Author contributions:
Qianming Bai: Design and conduct of the study, data collection and analysis, data interpretation and manuscript writing.
Xin Zhang: Conduct of the section of immunohistochemistry and animal treatments, and manuscript review.
Leyuan Xu: Conduct of the section of animal treatments.
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determined by Wako Reagents and hematoxylin and eosin (H&E) staining. Gene expressions were determined by real-time RT-PCR and Western Blot.

Results—Following infection, SULT2B1b was successfully overexpressed in liver, aorta and lung tissues, but not in heart or kidney. SULT2B1b overexpression, combined with administration of 25HC, significantly increased the formation of 25HC3S in liver tissue; significantly decreased serum and hepatic lipid levels, including triglycerides, total cholesterol, free cholesterol, and free fatty acids, as compared to controls both in C57BL/6 and $LDLR^{-/-}$ mice. Gene expression analysis showed that increases in SULT2B1b expression were accompanied by reduction in key regulators and enzymes involved in lipid metabolism, including LXRα, SREBP-1, SREBP-2, acetyl-CoA carboxylase-1 (ACC1) and fatty acid synthase (FAS).

Conclusion—These findings support the hypothesis that 25HC3S is an important endogenous regulator of lipid biosynthesis. This pathway may represent a novel target for pharmacological intervention in NAFLD.

Keywords

cytosolic sulfotransferase (SULT2B1b); oxysterol; oxysterol sulfation; 5-cholesten-3β-25-diol-3-sulfate (25HC3S); 25-hydroxycholesterol (25HC); lipid metabolism; nonalcoholic fatty liver disease (NAFLD)

Introduction

Nonalcoholic fatty liver disease (NAFLD) is emerging as the most common liver disease in the United States and worldwide [1, 2]. The spectrum of NAFLD ranges from simple non-progressive steatosis to progressive non-alcoholic steatohepatitis (NASH) that may lead to cirrhosis and hepatocellular carcinoma (HCC) [3–5]. The pathogenesis of steatosis and progression to NASH and cirrhosis involves numerous metabolic and inflammatory pathways and remains incompletely understood [6]. However, the central role of lipid accumulation in liver in the pathogenesis of NAFLD has been confirmed in clinical correlation studies and animal models [6, 7]. Thus, decreasing serum and hepatic lipid levels is crucial to the prevention of NAFLD.

Recently, we found that overexpression of mitochondrial cholesterol delivery protein (StARD1) up-regulated bile acid synthesis and secretion via the “acidic” pathway initiated by cholesterol 27-hydroxylase (CYP27A1), and down-regulated cholesterol and fatty acid biosynthesis. A search for the possible mechanism of these regulatory effects led to the finding of a novel sulfated oxysterol, 5-cholesten-3β-25-diol-3-sulfate (25HC3S) [8–10]. Addition of 25HC3S in human and rat hepatocytes and human acute monocytic leukemia cell line (THP-1) derived macrophages decreased expression and processing of nuclear LXR and its target gene SREBP-1, and subsequently decreased intracellular levels of free fatty acids, cholesterol, and triglycerides. Furthermore, addition of exogenous 25HC3S increased cytosolic IκB expression, decreased nuclear NFκB, and subsequently decreased inflammatory responses in hepatocytes [11, 12]. The results suggest that 25HC3S could play an important role in preventing nonalcoholic steatohepatitis. However, the effects of endogenous 25HC3S *in vivo* on serum and hepatic lipid metabolism have not been investigated.

It is reported that 25HC3S is synthesized by cytosolic sulfotransferase, SULT2B1b, through sulfation of 25-hydroxycholesterol (25HC) [13, 14]. Moreover, the regulatory properties of 25HC are opposite to those of 25HC3S, increasing nuclear LXR and SREBP-1 protein levels, and increasing ACC1 and FAS mRNA levels [11, 12, 14]. Thus, SULT2B1b, by determining the balance between 25HC and 25HC3S, could play an important role in lipid

metabolism. SULT2B1b is expressed in hepatocytes and liver tissue, and highly regulated by duration of culture and insulin in primary rat hepatocytes [13, 15, 16]. Overexpression of SULT2B1b in primary human aortic endothelial cells decreases mRNA and protein levels of LXRα, ABCA1, SREBP-1c, ACC and FAS, and subsequently reduces cellular lipid levels [14]. The effects of SULT2B1b overexpression in the presence of 25HC are similar to those produced by direct addition of 25HC3S in cell culture [14].

In the present study, we further evaluated the effect of SULT2B1b on the lipid metabolism in serum and liver tissue *in vivo* in mouse NAFLD models. The results indicate that 25HC sulfation by SULT2B1b substantially decreases serum and hepatic lipid levels by inhibiting the LXR-SREBP-1c signaling pathway. This finding supports the hypothesis that 25HC3S is an important endogenous regulator of lipid biosynthesis. This pathway may represent a novel target for pharmacological intervention in NAFLD.

Materials and Methods

Animals and treatment

Eight-week-old female C57BL/6 mice were purchased from Charles River Laboratories (Cambridge, MA), and $LDLR^{-/-}$ mice were obtained from Jackson Laboratory (Sacramento, CA). Mice were hosted under a standard 12/12-hour light/dark cycle. Mice were fed with either a high-cholesterol diet (HCD, 3.1 Kcal/g, 2% cholesterol and 5.7% fat), or a high-fat diet (HFD, 4.5 Kcal/g, 0.2% cholesterol and 21.2% fat) for 10 weeks. Mice were then infected with recombinant adenovirus encoding CMV-driven SULT2B1b (1×10^8 pfu/mouse) through tail vein injection. The CMV-SULT2B1b was prepared as previously described [14]. Ad-CMV-β-Gal adenovirus was used as control. In addition, some mice were given an intraperitoneal injection of 25HC (0.01% w/w, about 0.2mg/mouse) 2 days after infection. Mice were sacrificed following an overnight fast 6 days after adenovirus infection. The detailed information for the animal groups was shown in Table 1. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the McGuire VA medical center.

Immunohistochemistry

Formalin-fixed liver tissues were processed for histological analyses and stained with hematoxylin and eosin (H&E). Briefly, deparaffinized 4 μm sections were stained with rabbit anti-SULT2B1b antibody (Abcam, USA). Immobilized antibodies were detected by the avidin–biotin–peroxidase technique (Vectastain ABC Kits, Vector Laboratories, UK). DAB was used as the chromogen, and haematoxylin was used as the nuclear counterstain.

Analysis of composition of sulfated oxysterols in liver tissue

Total lipids in liver tissue were extracted by the Folch method [17]. Briefly, 200 mg of mouse liver tissue was homogenized in 1 ml of PBS. 20 ml of chloroform:methanol (2:1, v/v) was added in the homogenates, sonicated for 1–2 hrs, and filtered, 4 ml of water and 100 μl of 1 M K_2CO_3 were added, mixed, and allowed to stand for about 3 hrs for the phase separation.

The water/methanol (upper) phase, which contains sulfated oxysterols, was evaporated under N_2 stream. The residue was re-suspended in 0.5 ml of methanol, 3.5 ml of water and 0.5 ml of NaOH (1N) by sonication, and the suspension was passed through a preconditioned Sep-Pak C18 cartridge (Waters, Milford, MA) to remove non-sulfated oxysterols. After successively washing the cartridge with 8 ml of water, 3.5 ml of 15% acetone and 8 ml of water again, the sulfated oxysterol fraction was eluted in 5 ml of 75% methanol, which was taken to dryness under N_2 stream below 40 °C. The extracts were then

hydrolyzed in 1 ml of sulfatase (2 mg/ml) at 37 °C for 4 hrs. De-conjugated oxysterols were extracted by Folch's partition (chloroform:methanol, 2:1) and the chloroform phase was taken to dryness.

The oxysterol samples thus obtained from the methanol/water were oxidized with cholesterol oxidase as previously described [18]. To the oxysterol sample dissolved in 50 μl of 2-propanol were added 450 μl of water, 50 μl of 1M potassium phosphate buffer (Kpi) and 1.5 ug of progesterone as an internal standard, and the resulting mixture was sonicated for 10 min. To the mixture 0.4 units of cholesterol oxidase in 50 μl of Kpi buffer was added and incubated at 37 °C for 1 h. 300 μl of methanol was added to stop the reaction and the products, enones, were extracted 3 times with 2 ml of hexane, and the extracts were evaporated under N_2 stream. The residue was redissolved in 150 μl of 5% isopropanol in hexane and 100 μl of the solution was subjected to the HPLC as described below.

HPLC analysis was conducted with Alliance 2695 separation module fitted with 2487 Dual λ absorbance detector (Waters, Milford, MA). The separation was carried out on an Ultraspere silica column (5 μm, 4.6 mm id × 250 mm; Beckman, Urbana, IL) and hexane:isopropanol:acetic acid (965:25:10, v:v:v) as an eluent at a flow rate of 1.3 ml/min. The column temperature was kept constant at 30 °C. The enones were monitored at 240 nm absorption.

Lipid levels in liver tissue and sera

Liver cholesterol and triglycerides were extracted and analyzed as previously described [14]. Briefly, mouse liver tissue, 100 mg, was homogenized in 1 ml of PBS. The lipids in the homogenates were extracted overnight with 9 ml of chloroform:methanol (2:1, v/v), sonicated for 1–2 hrs, and filtered. The extract, 100 μl, was evaporated to dryness and dissolved in 100 μl of isopropanol containing 10% Triton X-100 for cholesterol assay; dissolved in isopropanol for triglycerides assay; or in NEFA solution (0.5 g of EDTA-Na_2, 2 g of Triton X-100, 0.76 ml of 1N NaOH, and 0.5 g of sodium azide/L of H_2O, pH 6.5) for free fatty acids assay. Total and free cholesterol, triglycerides, and free fatty acids assays were performed according to the manufacturer's instructions.

For serum analysis, the lipid levels and the liver-specific cytosolic enzyme activities of alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST) in serum of mice were determined by clinical biochemistry laboratory blood assays at the VA Medical Center. The lipoproteins of cholesterol (VLDL, LDL, and HDL) were measured by gel filtration using high pressure liquid chromatography (HPLC) as previously described [19] with mild modifications. Briefly, serum was centrifuged at 2000 rpm for 2 min, and 100 μl of supernatant was subjected to HPLC with Pharmacia Superose 6HR 10/30 column using mobile phase, 154 mM NaCl, 0.1mM EDTA pH 8.0 at flow rate of 0.2 ml/min. Each fraction was collected starting at 20 min, 1.2 min/each (240 μl) for up to 100 min. 180 μl of each fraction was transferred to a 96 well plate, and 20 μl Wako total cholesterol kit 10 × reagent buffer was added. After incubating at 37 °C for 3 hrs in the dark, the OD was read at 595 nm for cholesterol assay. The protein profile was monitored at OD 280 nm as internal control.

Determination of gene expression involved in lipid metabolism

Nuclear and cytosolic proteins from mouse liver tissue were extracted according to the manufacturer's instructions. 20 μg nuclear extracts or 50 μg cytosolic proteins were loaded on 10% SDS-PAGE for detection of the specific proteins, including LXRα, SREBP-1, SREBP-2, ACC-1, FAS, and SULT2B1b, using Lamin B1 and β-actin as loading control for

nuclear and cytosolic proteins respectively. Western blot analysis was performed as previously described [20].

Total RNA in liver tissue was isolated by SV total RNA isolation kit (Promega, Wisconsin, WI) following the manufacturer's instructions. The relative mRNA levels were measured by real-time RT-PCR as previously described [20]. Quantitative normalization of cDNA in each sample was performed using GAPDH as an internal control. The primer sets used in these assays are shown in Supplemental Table 1.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Significance of differences was determined using Student's t test for unpaired samples. A value of $P<0.05$ was considered statistically significant.

Results

Effect of adenovirus infection on liver toxicity

Following infection with different amount of recombinant adenovirus, serum activities of ALP, ALT, and AST and the ratios of liver to body weight were determined at the day 6 as shown in supplemental Fig. 1A and supplemental Fig. 1B. The activities and the ratios did not significantly change as compared to Ad-β-Gal infected mice indicating that no liver injury was occurred when less than 1×10^8 pfu of Ad-SULT2B1b/mouse was used. Thus, the dose of 1×10^8 pfu/mouse was selected to study the effect of SULT2B1b on lipid metabolism. Under this condition, no liver toxicity was detected in mice fed with HCD or HFD (supplemental Fig. 1C).

SULT2B1b expression in different tissues after infection with Ad-SULT2B1b

Mice were infected with Ad-SULT2B1b or Ad-control through tail vein injection in the condition as described above. Immunohistochemistry analysis showed that SULT2B1b gene expression following infection was significantly increased in liver, aorta and lung tissues, but not in heart or kidney (Fig. 1A). Consistently, western blot analysis following Ad-SULT2B1b infection showed that SULT2B1b gene expression increased by 20-fold in liver, with more modest increases of 1.5-fold in aorta and 2-fold in lung, as compared to control. No changes in expression of SULT2B1b were detected in heart or kidney (Fig. 1B and Fig. 1C).

Effect of SULT2B1b on oxysterols and sulfated oxysterols in liver tissues

To determine the specific activities of SULT2B1b, oxysterols and sulfated oxysterols were extracted from liver tissues and analyzed by HPLC. The results showed that SULT2B1b overexpression in the presence of 25HC significantly increased sulfated oxysterols, especially 25HC3S (Fig. 1E and Fig. 1G); decreased non-sulfated oxysterols, including 7-ketocholesterol (7KC), 6β-hydroxycholesterol (6βHC), and 25HC (Supplemental Fig. 2B and Supplemental Fig. 2D). However, in the absence of 25HC, SULT2B1b overexpression alone did not significantly change the levels of oxysterols and sulfated oxysterols (Fig. 1D, Fig. 1F, Supplemental Fig. 2A and Supplemental Fig. 2C).

Effect of SULT2B1b overexpression on lipid levels in sera and liver tissue

To study the effect of SULT2B1b on serum and hepatic lipid levels, C57BL/6 and $LDLR^{-/-}$ mice were infected by SULT2B1b adenovirus, with or without intraperitoneal injection of 25HC, as described above. Total cholesterol and triglycerides in sera were measured by clinical laboratory as shown in Table 2. Following the combination of SULT2B1b

overexpression and 25HC administration, triglyceride levels in serum in C57BL/6 mice_HCD were significantly decreased by 18% as compared to control mice injected with β-Gal virus. No significant change was observed in the absence of 25HC. In $LDLR^{-/-}$ mice_HFD, triglyceride levels in serum were decreased by 32% following SULT2B1b overexpression as compared to the control injected with β-Gal virus. However, total serum cholesterol levels both in C57BL/6 and $LDLR^{-/-}$ mice were unchanged with SULT2B1b overexpression. Interestingly, SULT2B1b overexpression decreased cholesterol levels in VLDL and LDL fraction in C57BL/6 and $LDLR^{-/-}$ mice while increased cholesterol levels in HDL fraction in C57BL/6 mice (Fig. 2A, Fig. 2B, and Fig. 2C). The protein profile (internal control) was unchanged following SULT2B1b overexpression (Fig. 2D, Fig. 2E, and Fig. 2F). These effects were much stronger in the presence of 25HC (Fig. 2).

Quantitative analysis of hepatic lipid levels showed that overexpression of SULT2B1b significantly decreased hepatic triglyceride, total cholesterol, and free cholesterol levels in the presence of 25HC in C57BL/6 mice_HCD (Fig. 3B). No significant change was detected in the absence of 25HC as compared to control (Fig. 3A). In $LDLR^{-/-}$ mice_HFD, SULT2B1b overexpression significantly decreased total cholesterol and free fatty acids levels but did not change triglycerides and free cholesterol levels significantly (Fig. 3C). Consistently, H&E staining also showed that SULT2B1b overexpression substantially decreased total neutral lipids in liver tissue both in C57BL/6 mice and $LDLR^{-/-}$ mice (Fig. 3D).

Effect of SULT2B1b on gene expressions involved in lipid metabolism

To better understand the mechanism of effects of SULT2B1b on lipid metabolism, gene expressions involved in lipid metabolism were determined. As expected, overexpression of SULT2B1b significantly decreased LXRα and SREBP-1 in nuclear protein levels but not SREBP-2 both in C57BL/6 mice and $LDLR^{-/-}$ mice fed with HCD or HFD. Consistently, SULT2B1b overexpression also significantly decreased the cytosolic protein levels of FAS and ACC1 as shown in Fig. 4A and Fig. 4B.

Real-time RT-PCR analysis of the gene expressions at mRNA level involved in lipid metabolism was shown in Table 3. Consistent with protein levels, SULT2B1b overexpression significantly decreased mRNA levels of LXRα, SREBP-1, SREBP-2, GPAM, ACAT2, CYP27A, ABCA1, ABCG1 and STS involved in lipid metabolism in the mice injected peritoneally with 25HC; in the absence of 25HC, SULT2B1b overexpression only decreased mRNA levels of LXRα, SREBP-1, and ABCA1.

Discussion

The present study shows that *in vivo* SULT2B1b overexpression with 25HC supplementation in mouse NAFLD models increases 25HC sulfation and levels of its product 25HC3S, mainly in liver tissue. These effects are accompanied by decreases in serum and hepatic triglycerides, total cholesterol, free cholesterol, and free fatty acids, accompanied by reduction in key regulators and enzymes in lipid metabolism, including SREBP-1, SREBP-2, acetyl-CoA carboxylase-1, and fatty acid synthase. The results are consistent with previous *in vitro* studies, demonstrating that SULT2B1b overexpression in the presence of 25HC leads to 25HC3S formation and decreases intracellular lipid levels [11, 12, 14]. The present results *in vivo* confirm that oxysterol sulfation by SULT2B1b can play an important regulatory role in lipid metabolism, and that oxysterol sulfation must be added to the list of signaling pathways involved in lipid homeostasis [14, 21]. These findings also suggest that 25HC3S may represent a novel strategy for therapy of NAFLD.

There is no significant difference in dietary intake between experimental and control mice (data not shown). Thus, serum and hepatic lipid levels depend on lipid synthesis and clearance. To confirm that the decreases in serum and hepatic lipid levels are mediated through suppression of lipid biosynthesis, low-density-lipoprotein receptor (LDLR) knockout mice have been used in the present study. LDLR is an important molecule in clearance of lipids from circulation [22–24]. Binding of lipoproteins to LDLR facilitates their endocytosis and delivery of lipids to target tissues [25]. Therefore, in this animal model, the serum lipid levels more directly represent the rates of lipid biosynthesis from liver tissue. Interestingly, overexpression of SULT2B1b in the knockout mice not only decreases neutral lipid levels in the liver but also changes the lipid profiles in the sera, indicating that SULT2B1b overexpression decreases VLDL and LDL synthesis and secretion from liver tissue and increases HDL synthesis (Fig. 2). HDL plays a key role in reverse cholesterol transport (RCT), which involves transport of cholesterol from peripheral tissues and cells to the liver, transforming it into bile acids, and finally eliminating it from the body [26–28]. It has been shown that the patients with high VLDL and LDL, low HDL are at a higher risk of heart attack and sudden death [29–32]. Whether these patients have lower expression of SULT2B1b or lower sulfated oxysterol levels is unknown, and merits further investigation.

Three SULT isoenzymes in human and four in rat have been cloned and identified [33–36]. He et al. reported that SULT2B1b has been localized to the cytosol and nuclei of both human cells and tissues [37]. Fuda et al. and Javitt et al. reported that SULT2B1b could sulfonate a variety of oxysterols, including 7-KC [33, 38]. Cells with high expression of SULT2B1b are significantly more resistant to the cytotoxic effects of 7KC, and overexpression of SULT2B1b protects from 7KC-induced loss of cell viability [38]. Our previous studies also showed that SULT2B1b effectively sulfates 25HC in human aortic endothelial cells, with the main product of 25HC3S [14]. However, in the present study, overexpression of SULT2B1b *in vivo* only significantly increased 25HC3S while other sulfated oxysterols such as 24HC-S, 27HC-S, 7KC-S, 6βHC-S, 7αHC-S, and 7βHC-S were almost unchanged or undetectable (Fig. 1D, Fig. 1E, Fig. 1F and Fig. 2G). These results suggest that SULT2B1b may have a potential specificity for 25HC sulfation in mouse liver. Furthermore, hepatic SULT2B1b expression in mice with high fat diet (HFD) decreased by 90% compared with those with normal cholesterol diet (NCD) or high cholesterol diet (HCD). Thus, we hypothesized that lower level of SULT2B1b may be one of the major pathogenesis during occurrence and development of NAFLD.

Overexpression of SULT2B1b inactivates the response of liver oxysterol receptor (LXR) to multiple oxysterol ligands [21], suggested that SULT2B1b is involved in LXR signaling pathway. It has been hypothesized that the oxysterol sulfation by SULT2B1b is an inactivation processing [21]. However, our laboratory studies showed that addition of exogenous 25HC3S to primary human hepatocytes and THP-1 derived macrophages, and overexpression of SULT2B1b in human aortic endothelial cells decrease SREBP-1/2 expression and block the activation of SREBP-1c; suppress the expression of key enzymes, including 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), ACC-1, and FAS involved in lipid metabolism; and subsequently decrease cholesterol and neutral lipid levels [11, 12, 14]. These results indicate that the sulfated oxysterol may act as an LXR antagonist rather than only an inactive form of LXR ligand and that SULT2B1b plays an important role in lipid homeostasis.

In conclusion, the present study adds to the convincing body of evidence implicating the oxysterol sulfate 25HC3S in regulation of serum and hepatic lipid metabolism *in vivo*. Oxysterol sulfation is catalyzed by SULT2B1b, and activity of this enzyme, by determining the balance between 25HC and 25HC3S, may play an important role in lipid homeostasis.

This pathway may represent a novel target for pharmacological intervention in lipid metabolic disorder related diseases, including NAFLD and atherosclerosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

SULT2B1b	cytosolic sulfotransferase
NAFLD	nonalcoholic fatty liver disease
25HC	25-hydroxycholesterol
25HC3S	5-cholesten-3β-25-diol-3-sulfate
LXR	liver X receptor
SREBPs	sterol regulatory element binding proteins
HCD	high cholesterol diet
HFD	high fat diet
ACC1	acetyl-CoA carboxylase-1
FAS	fatty acid synthase
StARD1	steroidogenic acute regulatory protein
CYP27A1	cholesterol 27-hydroxylase
ALP	alkaline phosphatase
ALT	alanine transaminase
AST	aspartate transaminase
VLDL	very low density lipoprotein
LDL	low density lipoprotein
HDL	high density lipoprotein
LDLR	low-density lipoprotein receptor
HPLC	high pressure liquid chromatography
7KC	7-ketocholesterol
6βHC	6β-hydroxycholesterol
PPAR	peroxisome proliferator activated receptor
ABCA1	ATP-binding cassette transporter A1
ABCG1	ATP-binding cassette transporter G1

HMGR	5-hydroxy-3-methylglutaryl-coenzyme A reductase
FABP4	fatty acid binding protein 4
FATP	fatty acid transport protein
GPAM	glycerol-3-phosphate acyltransferase, mitochondrial
MTTP	microsomal triglyceride transfer protein
PLTP	phospholipid transfer protein
ACAT	acetyl-coenzyme A acetyltransferase
STS	steroid sulfatase
TG	triglyceride
TC	total cholesterol
FC	free cholesterol
FFA	free fatty acid

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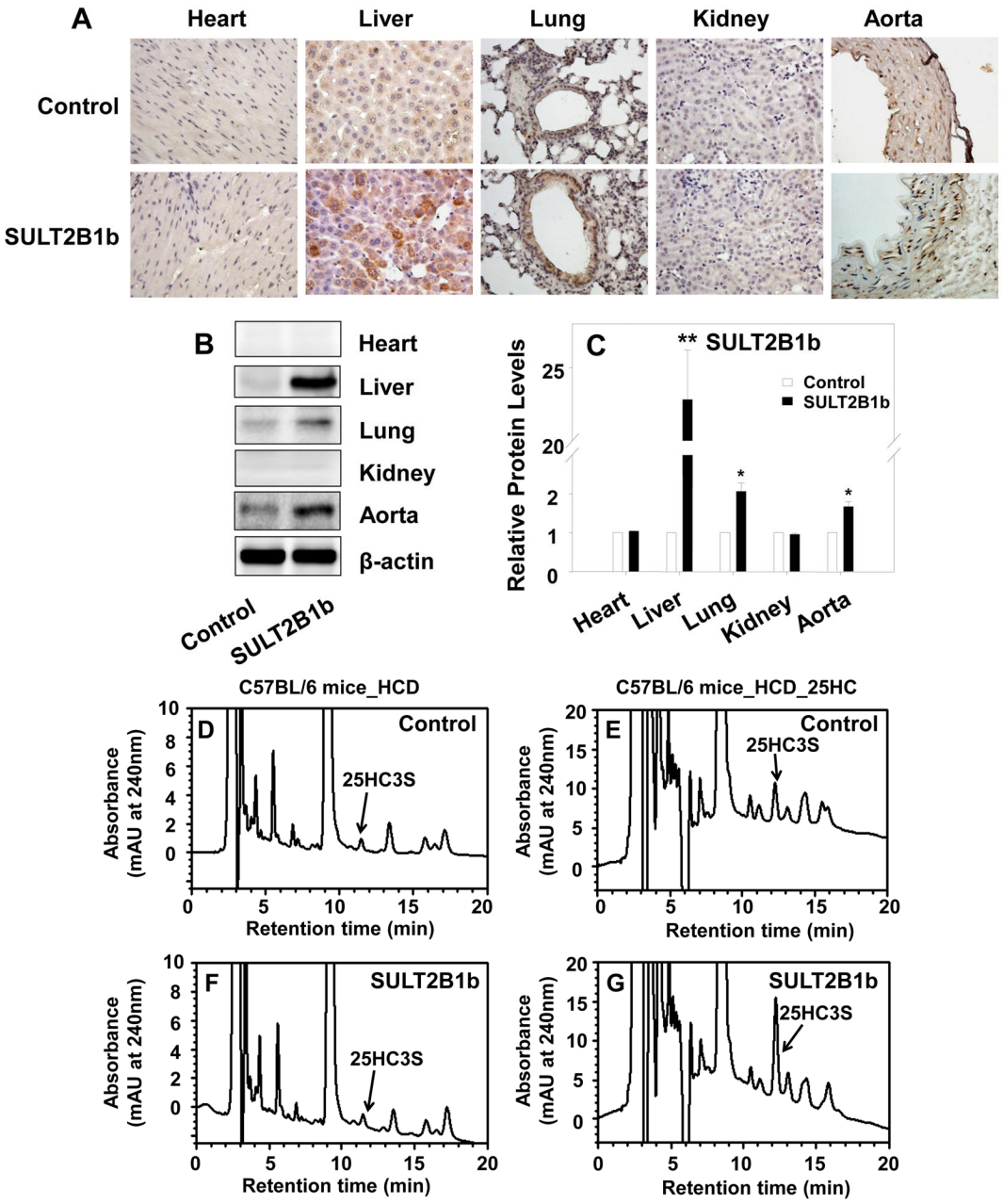


Fig. 1.

Determination of SULT2B1b expression in different tissues and SULT2B1b activities in liver tissue following infection with Ad-SULT2B1b. C57BL/6 mice, 8w, were fed with high cholesterol diet (HCD) for 10 weeks, and infected with Ad-control or Ad-SULT2B1b (1×10^8 pfu) in the presence or absence of 25HC as indicated. SULT2B1b protein expressions in different tissues were analyzed by immunohistochemistry at day 6 following infection (**A**) and its protein levels were analyzed by Western blot (**B** and **C**). Total intracellular lipids were extracted with chloroform/methanol. Sulfated oxysterols in the liver infected with Ad-β-Gal (**D** and **E**) or Ad-SULT2B1b (**F** and **G**) were analyzed by HPLC. 24-hydrocholesterol (24HC), 25-hydrocholesterol (25HC), 27-hydrocholesterol (27HC), 24, 25-epoxycholesterol (24, 25EC), 7-ketocholesterol (7KC), 6β-hydrocholesterol (6βHC), 7α-

hydrocholesterol (7αHC), and 7β-hydrocholesterol (7βHC) were used as standard controls. * P<0.05, ** P<0.01 vs. Control. N = 5 or 6.

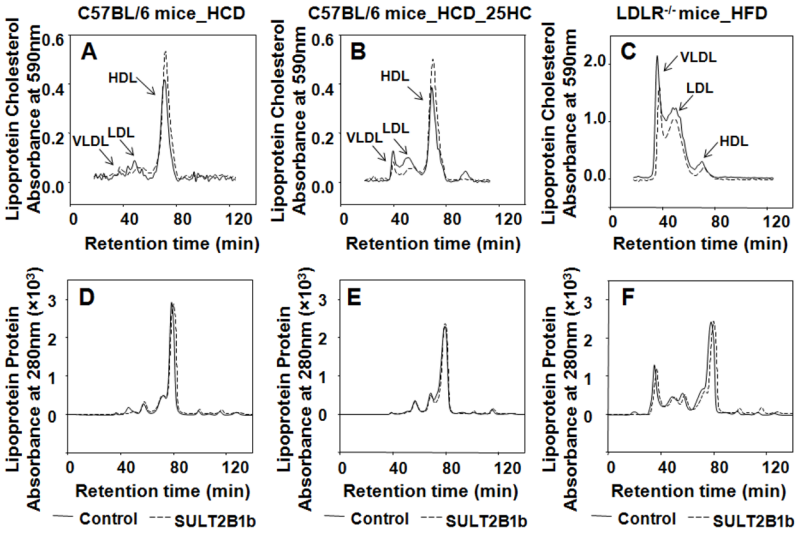


Fig. 2.

Effect of SULT2B1b overexpression on lipoprotein cholesterol in serum by HPLC. C57BL/6 mice and LDLR$^{-/-}$ mice, 8w, were fed with high cholesterol diet (HCD) or high fat diet (HFD) for 10 weeks, and then infected with Ad-control or Ad-SULT2B1b (1×10^8 pfu) in the presence or absence of 25HC as indicated. The lipoprotein (VLDL, LDL, and HDL) cholesterol levels in sera both in C57BL/6 mice and LDLR$^{-/-}$ mice were analyzed by HPLC (**A**, **B**, **C**). The lipoprotein protein levels were determined by absorbance at 280 nm (**D**, **E**, **F**). The data represent one of three separate experiments.

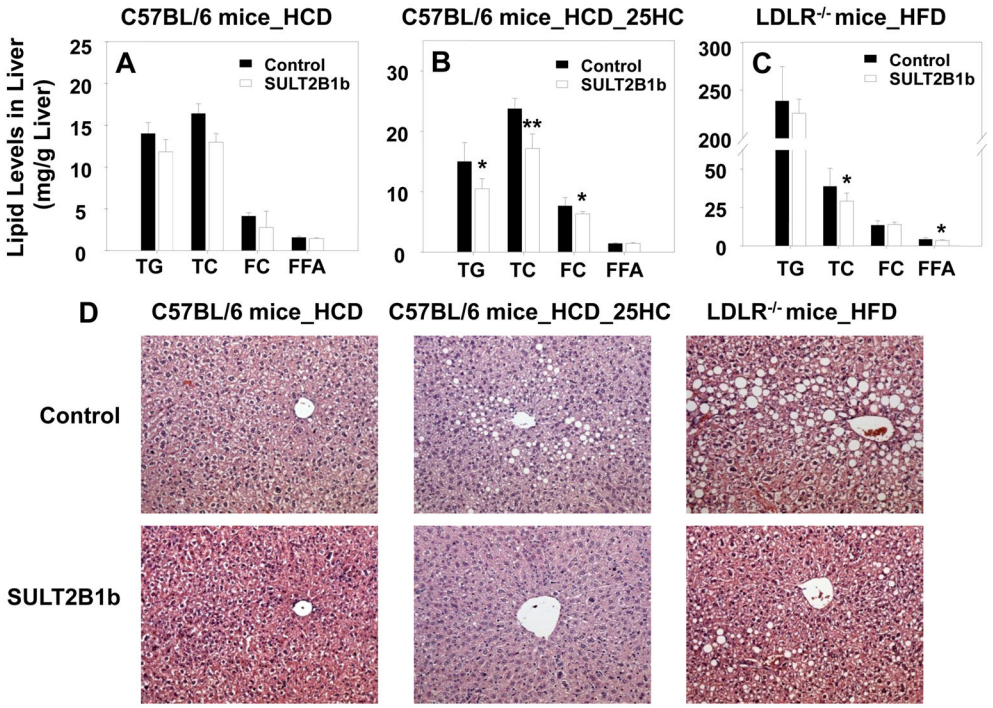
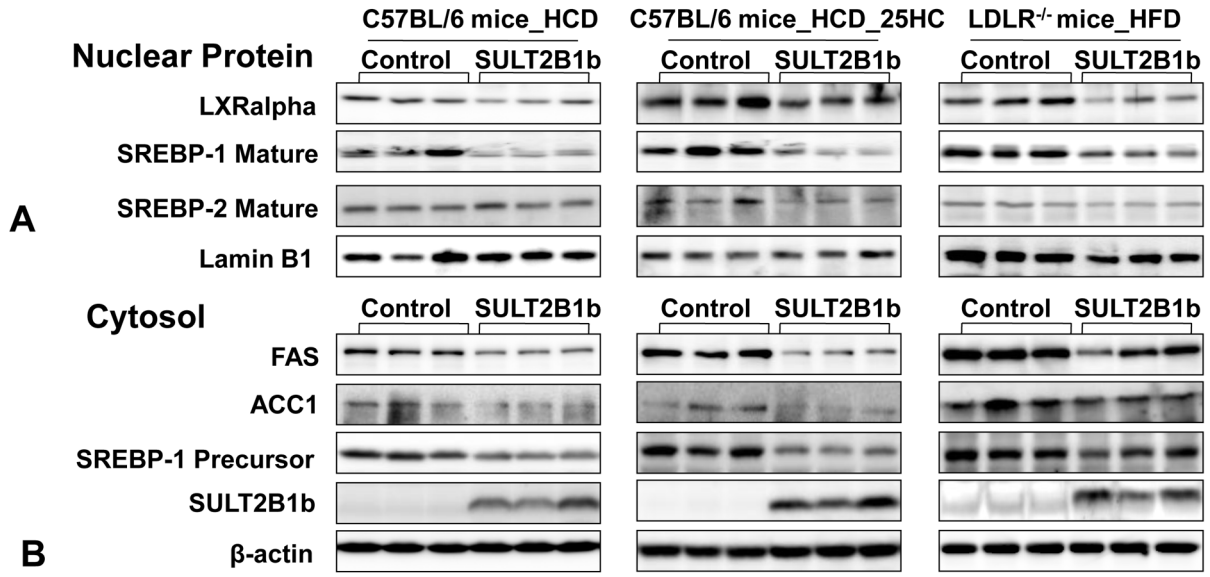


Fig. 3.

Effect of SULT2B1b overexpression on lipid levels in the liver tissues. The mice were fed and infected as stated in Fig. 2. Total intracellular lipids were extracted with chloroform/methanol. Triglycerides (TG), free fatty acids (FFA), total cholesterol (TC) and free cholesterol (FC) in liver both in C57BL/6 mice and $LDLR^{-/-}$ mice (**A**–**C**) were analyzed as described in Methods. Liver morphology was examined by H&E staining (**D**). * $P<0.05$, ** $P<0.01$ vs. Control. N = 5 or 6.



Relative hepatic protein expression in C57BL/6 mice or LDLR$^{-/-}$ mice after infection with β-Gal control or SULT2B1b virus

	C57BL/6 mice_HCD		C57BL/6 mice_HCD + 25HC		LDLR$^{-/-}$ mice_HFD	
	Control	SULT2B1b	Control	SULT2B1b	Control	SULT2B1b
Nuclear protein						
LXRalpha	1.0 ± 0.32	0.54 ± 0.19	1.0 ± 0.20	**0.68 ± 0.06 ***	1.0 ± 0.15	**0.51 ± 0.13 ****
SREBP-1 Mature	1.0 ± 0.23	**0.35 ± 0.07 ***	1.0 ± 0.38	**0.28 ± 0.11 ***	1.0 ± 0.15	**0.55 ± 0.07 ****
SREBP-2 Mature	1.0 ± 0.16	0.86 ± 0.03	1.0 ± 0.14	0.81 ± 0.17	1.0 ± 0.16	**0.65 ± 0.07 ***
Cytosol						
ACC-1	1.0 ± 0.34	**0.54 ± 0.13 ***	1.0 ± 0.20	**0.40 ± 0.03 ***	1.0 ± 0.35	0.81 ± 0.03
FAS	1.0 ± 0.11	**0.49 ± 0.02 ****	1.0 ± 0.18	**0.58 ± 0.14 ***	1.0 ± 0.08	**0.54 ± 0.17 ****
SREBP-1 Precursor	1.0 ± 0.15	**0.81 ± 0.01 ***	1.0 ± 0.21	**0.50 ± 0.07 ****	1.0 ± 0.09	**0.82 ± 0.10 ***
SULT2B1b	1.0 ± 0.10	**3.04 ± 0.45 ****	1.0 ± 0.07	**8.78 ± 2.43 ****	1.0 ± 0.08	**2.53 ± 0.78 ***

Values are mean ± SD; * $P < 0.05$, ** $P < 0.01$ vs Control

Fig. 4.

Effect of SULT2B1b overexpressoin on gene expressions involved in lipid metabolism at protein level. The mice were fed and infected as stated in Fig. 2. Nuclear proteins (LXRα, SREBP-1 mature, SREBP-2 mature) and cytosolic proteins (FAS, ACC1, SREBP-1 precursor, SULT2B1b) in liver tissue were analyzed by Western blot with specific antibodies (**A**). Western blot data were quantitatively analyzed (**B**). Cytoplamic proteins were normalized to β-actin; nuclear proteins, to Lamin B1. * $P<0.05$, ** $P<0.01$ vs. Control. N = 5 or 6.

Table 1

Animal groups

	C57BL/6 mice_HCD		C57BL/6 mice_HCD + 25HC		LDLR$^{-/-}$mice_HFD			
	Control	**SULT2B1b**	**Control**	**SULT2B1b**	**Control**		**SULT2B1b**	
Mouse Type	C57BL/6	C57BL/6	C57BL/6	C57BL/6	LDLR$^{-/-}$		LDLR$^{-/-}$	
Mouse Sex	F	F	F	F	F	M	F	M
Mouse Number	5	5	5	5	3	3	4	3
Diet	HCD	HCD	HCD	HCD	HFD		HFD	
Virus Infection	β-Gal	SULT2B1b	β-Gal	SULT2B1b	β-Gal		SULT2B1b	
IP inject 25HC	No	No	Yes	Yes	No		No	

HCD: high cholesterol diet, 2% cholesterol, 5.7% fat; HFD: high fat diet, 0.2% cholesterol, 21.2% fat; F: female; M: male.

Table 2

Lipids in serum in C57BL/6 mice or LDLR$^{-/-}$ mice after infection with β-Gal control or SULT2B1b virus

	C57BL/6 mice_HCD		C57BL/6mice_HCD + 25HC		LDLR$^{-/-}$mice_HFD	
	Control	**SULT2B1b**	**Control**	**SULT2B1b**	**Control**	**SULT2B1b**
TG (mg/dl)	53 ± 7.3	49 ± 12.4	39 ± 3.4	**32 ± 4.3** *	328 ± 92.8	**222 ± 47.2** *
TC (mg/dl)	117 ± 12.7	120 ± 8.4	102 ± 8.2	107 ± 9.3	1338 ± 203.8	1233 ± 155.6

Values are mean ± SD;

* P < 0.05 vs Control;

TG, triglycerides; TC, total cholesterol.

Table 3

Relative hepatic mRNA expression in C57BL/6 mice or LDLR$^{-/-}$ mice after infection with β-Gal control or SULT2B1b virus

	C57BL/6 mice_HCD		C57BL/6mice_HCD ± 25HC		LDLR$^{-/-}$mice_HFD	
	Control	**SULT2B1b**	**Control**	**SULT2B1b**	**Control**	**SULT2B1b**
Fatty acid metabolism						
SREBP-1c	1.0 ± 0.29	**0.57 ± 0.19** *	1.0 ± 0.16	**0.53 ± 0.18** **	1.0 ± 0.23	1.14 ± 0.27
ACC1	1.0 ± 0.37	0.93 ± 0.15	1.0 ± 0.27	0.79 ± 0.09	1.0 ± 0.22	0.84 ± 0.22
FAS	1.0 ± 0.41	0.76 ± 0.21	1.0 ± 0.50	0.57 ± 0.14	1.0 ± 0.27	1.01 ± 0.20
LXRa	1.0 ± 0.14	**0.81 ± 0.09** *	1.0 ± 0.30	**0.63 ± 0.08** *	1.0 ± 0.19	**0.82 ± 0.11** *
PPARa	1.0 ± 0.26	**0.59 ±0.17** *	1.0 ± 0.29	**0.58 ± 0.15** *	1.0 ± 0.27	**0.81 ± 0.19** *
FABP4	1.0 ± 0.51	068 ± 0.19	1.0 ± 0.45	**0.43 ± 0.16** *	1.0 ± 0.27	0.87 ± 0.27
FATP	1.0 ± 0.39	**0.59 ± 0.15** *	1.0 ± 0.25	**0.62 ± 0.17** *	1.0 ± 0.33	**0.51 ± 0.18** **
Triglyceride metabolism						
GPAM	1.0 ± 0.62	0.94 ± 0.36	1.0 ± 0.33	**0.69 ± 0.11** *	1.0 ± 0.19	0.90 ± 0.17
MTTP	1.0 ± 0.55	093 ± 0.41	1.0 ± 0.28	0.81 ± 0.16	1.0 ± 0.17	0.89 ± 0.16
PLTP	1.0 ± 0.69	0.94 ± 0.51	1.0 ± 0.13	0.85 ± 0.20	1.0 ± 0.15	**0.69 ± 0.11** **
Cholesterol metabolism						
SREBP-2	1.0 ± 0.68	0.92 ± 0.50	1.0 ± 0.08	**0.71 ± 0.07**	1.0 ± 0.14	0.94 ± 0.16
HMGR	1.0 ± 0.19	0.90 ± 0.08	1.0 ± 0.24	0.96 ± 0.24	1.0 ± 0.26	0.91 ± 0.29
LDLR	1.0 ± 0.20	1.13 ± 0.25	1.0 ± 0.39	**0.53 ± 0.11** *	1.0 ± 0.34	0.80 ± 0.22
ACAT1	1.0 ± 0.12	1.02 ± 0.35	1.0 ± 0.18	1.11 ± 0.29	1.0 ± 0.23	1.00 ± 0.13
ACAT2	1.0 ± 0.27	**0.61 ± 0.10** *	1.0 ± 0.21	**0.73 ± 0.06** *	1.0 ± 0.19	0.89 ± 0.10
Cholesterol efflux						
ABCA1	1.0 ± 0.22	**0.65 ± 0.09** *	1.0 ± 0.31	**0.51 ± 0.16** *	1.0 ± 0.26	0.89 ± 0.17
ABCG1	1.0 ± 0.23	1.15 ± 0.28	1.0 ± 0.29	**0.64 ± 0.22** *	1.0 ± 0.27	0.83 ± 0.19
Bile acid metabolism						
CYP7a	1.0 ± 0.33	0.78 ± 0.42	1.0 ± 0.35	0.57 ± 0.31	1.0 ± 0.20	0.90 ± 0.48
CYP27a	1.0 ± 0.27	0.80 ± 0.14	1.0 ± 0.31	**0.50 ± 0.07** *	1.0 ± 0.21	1.10 ± 0.21
Others						

	C57BL/6 mice_HCD		C57BL/6mice_HCD ± 25HC		LDLR$^{-/-}$mice_HFD	
	Control	**SULT2B1b**	**Control**	**SULT2B1b**	**Control**	**SULT2B1b**
SULT2B1b	1.0 ± 0.24	**193.5 ± 46.1** **	1.0 ± 0.17	**754.1 ± 114.8** **	1.0 ± 0.44	**324.6 ± 36.7** **
STS	1.0 ± 0.73	0.52 ± 0.23	1.0 ± 0.37	**0.41 ± 0.06** *	1.0 ± 0.57	1.28 ± 0.37

Values are mean ± SD; n = 5–6;

* P < 0.05,

** P < 0.01 vs Control.