Molecular Medicine

Cholesterol Esters (CE) Derived From Hepatic Sterol O-Acyltransferase 2 (SOAT2) Are Associated With More Atherosclerosis Than CE From Intestinal SOAT2

Jun Zhang, Janet K. Sawyer, Stephanie M. Marshall, Kathryn L. Kelley, Matthew A. Davis, Martha D. Wilson, J. Mark Brown, Lawrence L. Rudel

Rationale: Cholesterol esters (CE), especially cholesterol oleate, generated by hepatic and intestinal sterol O-acyltransferase 2 (SOAT2) play a critical role in cholesterol homeostasis. However, it is unknown whether the contribution of intestine-derived CE from SOAT2 would have similar effects in promoting atherosclerosis progression as for liver-derived CE.

Objective: To test whether, in low-density lipoprotein receptor null (LDLr−/−) mice, the conditional knockout of intestinal SOAT2 (SOAT2SI−/SI−) or hepatic SOAT2 (SOAT2L−/L−) would equally limit atherosclerosis development compared with the global deletion of SOAT2 (SOAT2−/−).

Methods and Results: SOAT2 conditional knockout mice were bred with LDLr−/− mice creating LDLr−/− mice with each of the specific SOAT2 gene deletions. All mice then were fed an atherogenic diet for 16 weeks. SOAT2SI−/SI−LDLr−/− and SOAT2−/−LDLr−/− mice had significantly lower levels of intestinal cholesterol absorption, more fecal sterol excretion, and lower biliary cholesterol levels. Analysis of plasma LDL showed that all mice with SOAT2 gene deletions had LDL CE with reduced percentages of cholesterol palmitate and cholesterol oleate. Each of the LDLr−/− mice with SOAT2 gene deletions had lower accumulations of total cholesterol and CE in the liver compared with control mice. Finally, aortic atherosclerosis development was significantly lower in all mice with global or tissue-restricted SOAT2 gene deletions. Nevertheless, SOAT2−/−LDLr−/− and SOAT2L−/−LDLr−/− mice had less aortic CE accumulation and smaller aortic lesions than SOAT2SI−/SI−LDLr−/− mice.

Conclusions: SOAT2-derived CE from both the intestine and liver significantly contribute to the development of atherosclerosis, although the CE from the hepatic enzyme appeared to promote more atherosclerosis development. (Circ Res. 2014;115:826-833.)

Key Words: atherosclerosis ■ cholesterol esters ■ molecular biology ■ sterol O-acyltransferase 2

Sterol O-acyltransferases (SOATs) are microsomal proteins responsible for catalyzing intracellular cholesterol ester (CE) synthesis using free sterol and acyl coenzyme A as substrates.1 SOAT subtype1 (SOAT1) is expressed in a variety of tissues while SOAT subtype2 (SOAT2) is expressed preferentially in enterocytes of the intestine and hepatocytes of the liver;2 where one of its roles is to generate CE for packaging into chylomicrons and very-low-density lipoproteins (VLDL), respectively.3,4 CE, especially cholesterol oleate, are major components of atherogenic lipoproteins, appearing to play a critical role in the pathogenesis of atherosclerosis.5,6

Previous studies have shown that SOAT2-deficient animals had delayed atherosclerosis development. Global deletion of SOAT2 in low-density lipoprotein receptor null (LDLr−/−) or apolipoprotein E null mice resulted in highly significant reductions of aortic atherosclerosis lesion area with a reduction in CE deposited in atherosclerotic plaques.7,8 These observed phenotypes are attributed to the combined effects of the decreased capability of cholesterol esterification and secretion by both the liver and the intestine, leading to a less atherogenic lipoprotein profile. However, some evidence has suggested that hepatic SOAT2 expression and activity in humans are different from that in rodents and nonhuman primates and might be expressed at a relatively lower level.9-11 Relevant to the human condition, it is uncertain whether blocking cholesterol absorption alone through inhibiting intestinal SOAT2 would be sufficient to delay atherosclerosis progression.
Through _LoxP-Cre recombinase_ technologies, our group previously generated 2 novel SOAT2 conditional knockout mice, the liver-specific (SOAT2<sup>L1–L3</sup>) and the intestine-specific (SOAT2<sup>SI–SI</sup>) knockouts. In response to increased dietary cholesterol, plasma and hepatic lipid changes of SOAT2<sup>SI–SI</sup> mice, indicated by low plasma VLDL cholesterol, low hepatic total cholesterol (TC), and CE concentrations, were essentially the same as occurred in SOAT2<sup>L1–L3</sup> mice. The data suggest that elimination of intestinal cholesterol esterification with subsequent absorption was sufficient to alleviate many of the dietary cholesterol-induced changes in cholesterol transport in the circulation and tissues.

Although the blunted cholesterol absorption that occurred via intestinal SOAT2 inhibition was hypocholesterolemic, the specific contribution made by intestine-derived CE to atherosclerosis development is still uncertain. LDLr<sup>−/−</sup> mice are known to develop a spectrum of cholesterol-enriched apolipoprotein B–containing lipoproteins, most of which apparently promote progression of atherosclerosis on high-fat/cholesterol-enriched diet. The mass and distribution of plasma lipids of LDLr<sup>−/−</sup> mice are marked and distinct from the relatively mild elevation of lipids after high cholesterol intake by wild-type mice. It is unknown whether, after cholesterol feeding, SOAT2<sup>SI–SI</sup>–LDLr<sup>−/−</sup> mice would be protected from atherosclerosis progression to the same extent as that of LDLr<sup>−/−</sup> mice with gene deletions of either hepatic SOAT2 or whole body SOAT2.

We think it is critically important to understand the tissue-specific roles for SOAT2 in atherosclerosis development if effective therapeutic strategies targeting SOAT2-driven cholesterol esterification are to be designed. This prompted us to cross tissue-specific SOAT2 knockouts with LDLr<sup>−/−</sup> mice. Here, we tested whether conditional knockout of hepatic SOAT2 versus intestinal SOAT2 similarly limit atherosclerosis development compared with the global deletion of SOAT2.

### Methods

#### Mice, Diet, and Study Design

Generation of tissue-specific SOAT2 knockout was described in detail previously. Mixed mice (Lox<sup>P</sup> sites flanked exons 11 through 13 of the SOAT2 gene on chromosome 15) are designated as SOAT2<sup>20</sup>. After introducing _Cre recombinase_ driven either by the _albumin_ or _villin_ promoter, SOAT2 was specifically deleted in the liver (SOAT2<sup>20Alb<sup>fl/fl</sup></sub>) or small intestine (SOAT2<sup>20Vil<sup>fl/fl</sup></sub>), which are designated as SOAT2<sup>20Alb<sup>−/−</sup></sub> or SOAT2<sup>20Vil<sup>−/−</sup></sub>. SOAT2<sup>20</sup> mice were maintained on a mixed background (strains of C57BL/6, 129S6, 129SvEv). To create the conditional knockouts on LDLr<sup>−/−</sup> background, SOAT2<sup>−/−</sup> and SOAT2<sup>20Alb<sup>−/−</sup></sub> mice were bred with SOAT2<sup>−/−</sup>LDLr<sup>−/−</sup> (strain of C57BL/6). Genotypes of litters were screened by polymerase chain reaction. Animals used in the study were from breeders set up as follows: heterozygotes of SOAT2<sup>20Alb<sup>−/−</sup></sub>LDLr<sup>−/−</sup> with or without Alb<sup>fl/fl</sup> (or Vil<sup>fl/fl</sup>) and SOAT2<sup>20Vil<sup>−/−</sup></sub>LDLr<sup>−/−</sup> with or without Alb<sup>fl/fl</sup> (or Vil<sup>fl/fl</sup>). Both male and female mice were included in the study. At the age of 8 to 9 weeks, mice were fed a semisynthetic diet containing fat (as lard) at 20% energy, protein 17% energy, carbohydrate 63% energy, and cholesterol 0.1% (wt/wt) for a total of 16 weeks. Blood was drawn at baseline, 8 weeks, and 16 weeks from mice that were fasted at 9 AM with blood collection from the superficial temporal vein starting at 1 PM. After consuming the diet for 8 weeks, mice were housed individually on wire bottom cages for 3 days to allow fecal collections for measurement of cholesterol absorption and fecal sterol loss. For necropsy at the end of the study, mice were fasted from 9 AM with terminations starting at 1 PM. All mice used in the studies were housed in a pathogen-free barrier facility at Wake Forest University School of Medicine approved by the American Association for Accreditation of Laboratory Animal Care. The Institutional Animal Care and Use Committee approved all protocols for use of animals before execution of the studies.

#### Plasma Lipid and Lipoprotein Analysis

Total plasma cholesterol (TPC) and triglyceride concentrations were measured using colorimetric enzymatic assays as previously described. For lipoprotein measurement, an aliquot of plasma containing 20 µg of TPC was diluted in PBS into a final volume of 400 µL. After centrifugation to remove any protein precipitates, samples were injected onto a Superose 6 HR 10/30 (Amersham Pharmacia) chromatography column, which was subsequently run at 0.4 mL/min. The signal was integrated using Chrom Perfect Spirit Software (Justice Laboratory Software). VLDL-, LDL-, and high-density lipoprotein cholesterol were determined by multiplying the TPC concentration by the cholesterol percentage within the elution region for each lipoprotein class.

#### LDL Isolation and CE Analysis

For isolated LDL, a narrow center of the LDL peak window (26–34 minutes) was collected for each sample. Total lipid of this LDL fraction was extracted with 6 mL chloroform/methanol (2:1), and phases were split with 0.7 mL H<sub>2</sub>O. After vortexing and centrifugation, the chloroform phase was recovered and dried down under nitrogen. The radioactivity was quantified in a liquid scintillation spectrometer.

#### Cholesterol Absorption Measurement by a Fecal Dual-Isotope Technique

The method was essentially as reported earlier by Temel et al. After 8 weeks of diet consumption, all mice were gavaged with 50 µL soybean oil containing 0.055 µCi of [14C]-cholesterol (American Radiolabeled Chemicals) and 0.135 µCi of [3H]-sitosterol (New England Nuclear) and were subsequently individually housed in wired-bottom cages with free access to food and water. Feces were collected for a total of 3 days and dried overnight in a vacuum oven at 70°C. Dried fecal pellets were crushed into fine powder, and ~50 mg of fecal samples, along with 100 µg 5-α-tetrol cholestane (as an internal standard), were saponified and extracted with hexane. For fractional cholesterol absorption, an aliquot of hexane phase was dried under nitrogen. The radioactivity was quantified in a liquid scintillation spectrometer (Beckman Coulter LS 6500), and percentage of cholesterol absorption was calculated as 100 × ([14C]/[3H] in dose –[14C]/[3H] in feces)/([14C]/[3H] in dose). Another aliquot of hexane phase was transferred to a gas-liquid chromatography vial, and fecal neutral sterol (FNS) was quantified by gas-liquid chromatography. The mass of FNS represented the sum of cholesterol and coprosterol, and results were expressed as mg sterol per day per 100 g body weight.
Hepatic and Biliary Lipid Analysis

Extraction of liver and biliary lipids for enzymatic quantification of total triglyceride, CE, free cholesterol (FC), phospholipids and bile acids were performed as previously described. Briefly, for analysis of the liver lipids ≤50 to 100 mg of liver sample was minced and thawed in a glass tube. Lipids were extracted in chloroform/methanol (2:1) at room temperature overnight. Lipid extract was dried down under nitrogen and redissolved in a measured volume of 2:1 chloroform/methanol. After addition of 0.05% H2SO4 and centrifugation, the aqueous upper phase was aspirated and discarded, and an aliquot of the bottom phase was transferred and dried down; 1% Triton X-100 in chloroform was then added, and the solvent was evaporated. Deionized water was then added to each tube and vortexed until the solution was clear. Lipids were quantified using available enzymatic assay kits. For analysis of biliary lipid concentrations, a measured volume (5–10 µL) of bile was placed into a glass tube and the lipids were extracted in chloroform/methanol (2:1). An aliquot of chloroform phase was used for quantification of biliary cholesterol and phospholipids, similarly as described for liver lipid measurement. An aliquot of the aqueous phase of the extraction was analyzed for total bile acid content using hydroxysteroid dehydrogenase-based enzymatic assay.

Quantification (En Face) of Aortic Lesion

At the time of necropsy, the entire length of aorta was excised and submersion fixed in 10% formalin for ≥24 hours. Adherent adipose and connective tissue were removed before the measurement. The aortas were opened longitudinally along the ventral midline and then pinned flat. Images of the aortas were captured using a Sony digital camera (Model DXC-S500) and evaluated for lesion extent using Scion Image software (Version 1.62). Percent of surface area occupied by lesion was calculated and expressed as percentage of surface area with visible atherosclerotic lesion.

Quantification of Cholesterol in the Aortic Plaques

Lipids of the fixed entire aorta were then extracted in chloroform/methanol (2:1) overnight with addition of 20 µg of 5α-cholestanet as an internal standard. Aortic protein was then washed twice with chloroform/methanol (2:1), and pooled solvent with all lipids was evaporated down to 250 µL hexane, and 1 µL of hexane phase was injected onto ZB-50 gas-liquid chromatography column to measure FC. After the FC quantification, the remaining samples were saponified and TC of aortas was also determined by gas-liquid chromatography. Aortic CE was calculated using the equation (TC–FC)/1.67.

Statistical Analyses

All graphs were plotted using GraphPad Prism (version 5.05) or Microsoft Excel for Mac (version 14.4.3). Data were analyzed by 1-way or 2-way ANOVA with Tukey post hoc test using GraphPad Prism (version 5.05) or JMP statistical software (version 5.0.1.2). Relationships between lipid and atherosclerosis parameters were analyzed by regression analysis with associations shown with least-square best-fit regression lines. Regression coefficients are given in the figures. Statistically significant differences were considered at P<0.05.

Results

Intestinal SOAT2, But Not Liver SOAT2, Is a Critical Determinant of Cholesterol Absorption

SOAT2 wild-type control mice (SOAT2+/+LDLr−/−) and SOAT2 floxed mice (SOAT2fl/flLDLr−/−) had similar levels of cholesterol absorption (Figure 1A). In agreement with our previous findings, SOAT2 total body knockout mice (SOAT2L−/L−LDLr−/−) and intestinal SOAT2 knockout mice (SOAT2fl/flL−/−LDLr−/−) had significantly decreased levels of cholesterol absorption, compared with SOAT2+/+LDLr−/− control mice with their level of decrease being 33% and 35%, respectively (Figure 1A). Cholesterol absorption in liver-specific SOAT2 knockout mice (SOAT2−/−LDLr−/−) did not differ significantly from that of SOAT2+/+LDLr−/− mice (Figure 1A). SOAT2+/−LDLr−/− mice had 2-fold more fecal neutral sterol loss, compared with control mice (Figure 1B). Total body SOAT2−/−LDLr−/− mice also showed significantly more FNS excretion (2.9-fold), compared with SOAT2+/−LDLr−/− control mice (Figure 1B). The amount of fecal neutral sterol loss of SOAT2+/−LDLr−/− mice was similar to that of floxed control mice, and this outcome was the same as for FNS excretion in the LDLr−/− mice with SOAT2 intact in the enterocytes of the small intestine (Figure 1B).

Hepatic SOAT2, But Not Intestinal SOAT2, Determines LDL-Cholesterol Levels in LDLr−/− Mice

TPC concentrations were similar among the various genotypes at the beginning of the study (week 0, Table 1). LDLr−/− mice with SOAT2 intact had significant time-dependent increases of TPC after 8 and 16 weeks of consumption of the cholesterol-enriched diet (Table 1). SOAT2+/−LDLr−/− and SOAT2+/−LDLr−/− mice had similar levels of TPC as the LDLr−/− mice with SOAT2 intact at each time point. By contrast, at the end of weeks 8 and 16, TPC concentrations were significantly lower in LDLr−/− mice with SOAT2+/− and SOAT2+/− genotypes (Table 1).

All groups of LDLr−/− mice had similar levels of plasma triglyceride at week 0. Plasma triglyceride of SOAT2+/+LDLr−/− and
Table 1. Plasma Total Cholesterol and Triglycerides

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Time</th>
<th>+/+</th>
<th>fl/fl</th>
<th>−/−</th>
<th>L−/−</th>
<th>SI−/SI−</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>wk 0</td>
<td>214±7</td>
<td>206±9</td>
<td>119±4</td>
<td>153±8</td>
<td>163±7</td>
</tr>
<tr>
<td></td>
<td>wk 8</td>
<td>633±15</td>
<td>604±14</td>
<td>333±20</td>
<td>413±23</td>
<td>534±29</td>
</tr>
<tr>
<td></td>
<td>wk16</td>
<td>741±31</td>
<td>689±25</td>
<td>443±26</td>
<td>478±31</td>
<td>685±44</td>
</tr>
<tr>
<td>TG</td>
<td>wk 0</td>
<td>84±5</td>
<td>67±5</td>
<td>87±3</td>
<td>85±5</td>
<td>86±7</td>
</tr>
<tr>
<td></td>
<td>wk 8</td>
<td>160±8</td>
<td>133±7</td>
<td>216±25</td>
<td>204±13</td>
<td>303±40</td>
</tr>
<tr>
<td></td>
<td>wk16</td>
<td>213±21</td>
<td>155±16</td>
<td>412±39</td>
<td>360±33</td>
<td>532±59</td>
</tr>
</tbody>
</table>

Two-way ANOVA and Tukey post hoc analyses were applied to TC and separately, TG, for all groups at all times to determine the difference in genotype, time, and genotype by time interaction for either lipid. Superscripts within each lipid class (TC or TG) not sharing common letters differ with P<0.05 with all data included in the comparison. Data represent the mean (mg/dL)±SEM of 19 to 20 mice per genotype. +/+ indicates SOAT2+/+LDLr−/−; −/−, SOAT2−/−LDLr−/−; fl/fl, SOAT2fl/flLDLr−/−; L−/−, SOAT2L−/L−LDLr−/−; SI−/SI−, SOAT2SI−/SI−LDLr−/−; TC, total cholesterol; and TG, triglycerides.

SOAT2fl/flLDLr−/− mice increased by 2- to 2.5-fold after 16 weeks of being fed the elevated diet cholesterol level (Table 1). All mice with a SOAT2 gene deletion had time-dependent elevations of plasma triglyceride. Relative to the level at week 0, plasma triglyceride was significantly increased by 4.6-fold, 4-fold, and 6-fold in SOAT2−/−LDLr−/−, SOAT2L−/L−LDLr−/−, and SOAT2SI−/SI−LDLr−/− mice, respectively, at the end of week 16 (Table 1). The increased level of plasma triglyceride that occurred together with cholesterol feeding was most pronounced in SOAT22-fl/−LDLr−/− mice but the explanation for this outcome is unknown.

To examine possible genetic responses that could be related to the hypertriglyceridemia in the SI−/SI− mice, we examined gene expression levels for several genes in liver and intestine of whole body and intestine-specific SOAT2 stearoyl-CoA desaturase 1 (SCD1) which was elevated over 10-fold in the livers of these animals. Understanding of relationship between intestinal SCD-1 and hypertriglyceridemia in mice with the livers of these animals. Further analysis indicated that the percentages of palmitic acid (C16:0), stearic acid (C18:0), palmitoleic acid (C16:1), and oleic acid (C18:1) all were significantly decreased in LDL CEs of mice with SOAT2 gene deletions compared with SOAT2+/+LDLr−/− mice, all LDLr−/− mouse groups of the study (Figure 3A). Compared with LDLr−/− mice with SOAT2 intact, all LDLr−/− mice with SOAT2 gene deletions had significantly lower levels of plasma VLDDL cholesterol, although this measurement in the mice with the intestine-specific SOAT2 deletion was as high as that in the floxed mice. SOAT2−/−LDLr−/− and SOAT2L−/L−LDLr−/− mice but not SOAT22-fl/−LDLr−/− mice had significantly lower LDL cholesterol concentrations, compared with the SOAT2 intact mice (Figure 2A). High-density lipoprotein cholesterol was not different among genotypes. Representative high-performance liquid chromatography chromatographic profiles of lipoprotein cholesterol from animals representing each study group are shown in Online Figure I.

CE compositions were also measured in plasma LDL of all LDLr−/− mouse groups of the study (Figure 2B; Table 2). Groups with SOAT2 gene deletions had significantly lower percentages of saturated and monounsaturated fatty acids and higher percentages of polyunsaturated fatty acids (Figure 2B). These differences were most remarkable in mice with no hepatic SOAT2 (ie, SOAT2−/−LDLr−/− and SOAT2L−/L−LDLr−/− mice).

Further analysis indicated that the percentages of palmitic acid (C16:0), stearic acid (C18:0), palmitoleic acid (C16:1), and oleic acid (C18:1) all were significantly decreased in LDL CEs (Table 2) while percentages of linoleic acid (C18:2), arachidonic acid (C20:4), eicosapentaenoic acid (C20:5), and docosahexaenoic acid (C22:6) all were increased in LDL CE of mice with SOAT2 gene deletions compared with LDL CE in LDLr−/− mice having intact SOAT2 (Table 2). The extent of the decrease in the proportion of monounsaturated CE (and increase in polyunsaturated CE) was intermediate in the LDL of SOAT22-fl/− mice.

Both Intestine- and Liver-Specific SOAT2 Knockout Mice Are Protected From Diet-Induced Hepatic Cholesterol Accumulation

Liver weight and body weight were not different among genotypes at the time of necropsy (Online Figure I). SOAT2−/−LDLr−/− and SOAT22-fl/−LDLr−/− mice had similar concentrations of each of the hepatic lipids including TC, PC, CE, and triglyceride (Figure 3). Compared with SOAT2−/−LDLr−/− mice, all SOAT2 knockouts had significant lower concentrations of liver TC and CE (Figure 3A and 3B). SOAT2−/−LDLr−/− and SOAT2L−/L−LDLr−/− mice had less TC and CE accumulated in the liver than SOAT22-fl/−LDLr−/− mice (Figure 3A and 3B). SOAT2−/−LDLr−/− mice also had significant less FC and triglyceride in the liver.
Critical Determinant of Biliary Cholesterol Levels

Intestinal SOAT2, But Not Liver SOAT2, Is a

Table 2. Percentage Fatty Acid Composition of LDL CE

<table>
<thead>
<tr>
<th>Fatty Acid Species</th>
<th>+/+</th>
<th>fl/fl</th>
<th>SOAT2 L−/L−</th>
<th>SOAT2 SI−/SI−</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>9.1±0.3</td>
<td>8.9±0.5</td>
<td>3.4±0.1 b</td>
<td>4.9±0.2 c</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.1±0.2</td>
<td>1.9±0.3</td>
<td>0.3±0.1 b</td>
<td>0.7±0.1 b</td>
</tr>
<tr>
<td>C16:1</td>
<td>12.8±1.0</td>
<td>13.3±1.2</td>
<td>3.6±0.5 b</td>
<td>5.3±0.5 b</td>
</tr>
<tr>
<td>C18:1</td>
<td>39.0±3.0</td>
<td>37.0±3.5</td>
<td>7.0±0.2 b</td>
<td>10.0±0.5 b</td>
</tr>
<tr>
<td>C18:2</td>
<td>18.5±1.5 a</td>
<td>20.0±2.3 a</td>
<td>36.5±1.0 b</td>
<td>35.2±1.4 a b</td>
</tr>
<tr>
<td>C18:3</td>
<td>1.7±0.3 a</td>
<td>1.4±0.3 a</td>
<td>2.0±0.4 a</td>
<td>2.0±0.4 a b</td>
</tr>
<tr>
<td>C20:4</td>
<td>13.5±1.0 a</td>
<td>13.8±1.3 a</td>
<td>37.8±1.6 b</td>
<td>33.4±1.7 a b</td>
</tr>
<tr>
<td>C20:5</td>
<td>1.4±0.2 a</td>
<td>1.5±0.3 a b</td>
<td>3.0±0.2 b</td>
<td>3.3±0.2 b</td>
</tr>
<tr>
<td>C22:6</td>
<td>2.0±0.2 a</td>
<td>2.0±0.4 a</td>
<td>5.3±0.1 b</td>
<td>4.9±0.2 a</td>
</tr>
</tbody>
</table>

Fatty acid species of LDL CE were quantified by LC-MS. Values are expressed as percentage of the mass of CE containing individual fatty acid to total mass of CE recovered in LDL. Data represent the mean±SEM from 9 to 10 samples per genotype. Superscripts not sharing common letters within each row indicate difference from SOAT2+/+LDLr−/− control mice with any of the genotypes (data not shown).

Percentage Fatty Acid Composition of LDL CE

Table 2.

- C16:0: 9.1±0.3, 8.9±0.5, 3.4±0.1, 4.9±0.2, 6.2±0.3
- C18:0: 2.1±0.2, 1.9±0.3, 0.3±0.1, 0.7±0.1, 0.9±0.2
- C16:1: 12.8±1.0, 13.3±1.2, 3.6±0.5, 5.3±0.5, 7.5±0.8
- C18:1: 39.0±3.0, 37.0±3.5, 7.0±0.2, 10.0±0.5, 24.5±1.5
- C18:2: 18.5±1.5, 20.0±2.3, 36.5±1.0, 35.2±1.4, 23.3±0.7
- C18:3: 1.7±0.3, 1.4±0.3, 2.0±0.4, 2.0±0.4, 1.6±0.2
- C20:4: 13.5±1.0, 13.8±1.3, 37.8±1.6, 33.4±1.7, 30.0±1.5
- C20:5: 1.4±0.2, 1.5±0.3, 3.0±0.2, 3.3±0.2, 2.0±0.1
- C22:6: 2.0±0.2, 2.0±0.4, 5.3±0.1, 4.9±0.2, 4.0±0.4

while SOAT2 conditional knockouts had similar hepatic FC and triglyceride as control mice (Figure 3C and 3D). The concentration of phospholipid (PL) was not significantly different among any of the genotypes (data not shown).

Intestinal SOAT2, But Not Liver SOAT2, Is a Critical Determinant of Biliary Cholesterol Levels

SOAT2+/−LDLr−/− and SOAT2+/−LDLr−/− mice had similar levels of biliary cholesterol (Online Figure III). By contrast, SOAT2+/−LDLr−/− and SOAT2+/−LDLr−/− mice had significantly less biliary cholesterol (Online Figure III). Both end points for LDL are significantly related to the extent of atherosclerosis, but the CE composition of LDL seems to be the more highly associated.

Regression analysis showed that the percentage of aortic surface area as lesion was significantly associated with aortic CE concentration (\(r=0.86, P<0.0001\); data not shown). Furthermore, the percentage of monounsaturated CEs in LDL was significantly correlated with aortic CE concentration (\(r=0.74, P<0.0001\); Online Figure IV) and with aortic surface area occupied with lesion (\(r=0.58, P<0.0001\); Figure 5C). The importance of LDL CE composition as a contributor to atherogenesis in addition to LDL cholesterol concentration can be appreciated when comparisons between the regressions of LDL cholesterol and LDL CE composition with atherogenesis (in this case expressed as aortic CE concentration) were made (Online Figure IV). Both end points for LDL are significantly related to the extent of atherosclerosis, but the CE composition of LDL seems to be the more highly associated.

Discussion

Previous work from our laboratory has consistently shown that mice with whole body SOAT2 gene deletions are protected from dietary hepatic cholesterol accumulation in the liver. Lipids of preweighed liver sample (50–100 mg) were extracted with CHCl3/MeOH (2:1). Quantification of hepatic lipids was determined by commercial enzymatic assay kits. A. Hepatic total cholesterol, B) cholesterol ester, C) free cholesterol, and \(D\) triglycerides. Data represent the mean±SEM from 19 to 20 mice per group and are expressed as mg lipids per wet weight. Bars not sharing common letters differ with \(P<0.05\).
against atherosclerosis development.\textsuperscript{7,8} Because \textit{SOAT2} is selectively expressed in only 2 tissues, the liver and the intestine,\textsuperscript{7,11} both of which are lipoprotein CE secreting organs, we felt that it would be informative to determine if there were differences in the effect on atherosclerosis of having \textit{SOAT2} deletions separately in either of these tissues. To achieve this, the \textit{SOAT2} deletions need to be expressed in an atherosclerosis-susceptible mouse model. Given the lipoprotein profile of LDL receptor–deficient versus apolipoprotein E–deficient mice, the 2 main mouse models of atherosclerosis susceptibility, we studied the LDL receptor-deficient mouse model because the lipoprotein profile is typically more similar to that seen in humans and is the mouse model that we have most often used previously. Our data show that although there is a somewhat higher level (≈2×) of \textit{SOAT2} activity in the intestine than in the liver in mice, the CEs secreted into lipoproteins by the liver are expected to more readily distribute into the LDL, which are the lipoproteins that appear to circulate longer at higher concentrations in plasma during atherogenesis. Thus, given that this study was done in LDL receptor–deficient mice, the outcome will be characteristic of mice with higher LDL levels. Our data show that although there is a somewhat higher level (≈2×) of \textit{SOAT2} activity in the intestine than in the liver in mice, the CEs secreted into lipoproteins by the liver are expected to more readily distribute into the LDL, which are the lipoproteins that appear to circulate longer at higher concentrations in plasma during atherogenesis. Thus, given that this study was done in LDL receptor–deficient mice, the outcome will be characteristic of mice with higher LDL levels. Our data show that although there is a somewhat higher level (≈2×) of \textit{SOAT2} activity in the intestine than in the liver in mice, the CEs secreted into lipoproteins by the liver are expected to more readily distribute into the LDL, which are the lipoproteins that appear to circulate longer at higher concentrations in plasma during atherogenesis. Thus, given that this study was done in LDL receptor–deficient mice, the outcome will be characteristic of mice with higher LDL levels. Our data show that although there is a somewhat higher level (≈2×) of \textit{SOAT2} activity in the intestine than in the liver in mice, the CEs secreted into lipoproteins by the liver are expected to more readily distribute into the LDL, which are the lipoproteins that appear to circulate longer at higher concentrations in plasma during atherogenesis. Thus, given that this study was done in LDL receptor–deficient mice, the outcome will be characteristic of mice with higher LDL levels.

Excessive circulating apolipoprotein B–containing lipoproteins, especially LDL, would be retained in the arterial intima\textsuperscript{6} and may initiate atherogenesis. \textit{SOAT2} knockout animals have delayed atherosclerosis development that is presumably because of the loss of capability of esterification of cholesterol oleate and packaging of those CEs into potentially atherogenic lipoprotein particles. Interestingly, intestine \textit{SOAT2} knockouts in the study had significantly less aortic lesion area and cholesterol deposition regardless of similar total mass of plasma LDL as that found in control mice (Figure 2). The small but significant decrease in VLDL cholesterol (and possibly reduced VLDL cholesterol oleate) in the \textit{SOAT2\textsuperscript{L−/L−}} mice may contribute to the decrease in atherosclerosis in these animals. One recent study suggested that LDL core enrichment in cholesterol oleate led to intimal

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image}
\caption{Images of aorta isolated from all sterol O-acyltransferase 2 knockouts. At the time of necropsy, the entire length of aorta was excised. Atherosclerotic lesions were noticeable as opaque areas mainly in the areas of ascending aorta and aortic arch. Representative image of each genotype was presented. \textit{N}=10 to 12 per genotype.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image}
\caption{All sterol O-acyltransferase 2 knockouts are protected from atherosclerosis progression. \textbf{A}, En face analysis of aortic surface lesion area. Data were expressed as percentage of lesion surface area to total aorta surface area. \textit{N}=10 to 12 per genotype. Scatterplots not sharing common letters differ with \textit{P}<0.05. \textbf{B}, Lipids of entire aorta were extracted with CHCl\textsubscript{3}/MeOH (2:1). Cholesterol was quantified by gas liquid chromatography. Data represent the mean±SEM from 14 to 16 mice per genotype. Statistical analyses were performed for each subclass of aortic lipids. Bars not sharing common letters differ with \textit{P}<0.05. \textbf{C}, Correlation was calculated between percentage of cholesterol oleate in low-density lipoprotein (LDL)-cholesterol ester (CE) and aortic CE using least-square regression model. FC indicates free cholesterol; and TC, total cholesterol.}
\end{figure}
deposition in the aorta through LDL-proteoglycan binding.6 LDL isolated from global SOAT2 knockouts had minimal cholesterol oleate in the core and had a lower affinity to bind to proteoglycan,5 which could contribute to the delayed atherosclerosis development. This study pinpoints the critical importance of LDL cholesterol oleate, in addition to LDL cholesterol mass (Online Figure IV), as a potential marker to predict the severity of the atherosclerosis. As discovered by Miller et al.,17 plasma cholesterol oleate and other SOAT2-derived CE are valuable in predicting a patient’s possibility of having acute coronary syndrome during an attack of sudden chest pain. Furthermore, SOAT2-derived CEs have recently been identified as predictors of cardiovascular disease risk in a large unbiased human lipodermic study.18 These results suggest that our current findings have broad implications in human disease. Our current study also greatly extends our understanding of distinct roles of liver- and intestine-derived CE in atherosclerosis progression. Although intestine-specific SOAT2 knockouts were protected from atherosclerosis development, protection was not to the extent seen in liver-specific SOAT2 and total body SOAT2 knockouts. The difference could be explained by the fact that intestinal knockouts still efficiently esterify cholesterol in the liver despite the reduction in intestinal cholesterol absorption. This speculation is supported by the fact that more cholesterol oleate was recovered in LDL-CE of SOAT2SI−/SI−LDLr−/− mice than other 2 knockouts.

One of the characteristic changes in plasma lipids was the significant increase of triglyceride in whole body SOAT2 knockout, antisense oligonucleotide-mediated hepatic SOAT2 knockdown, and liver-specific SOAT2 knockouts.5,14,16,19–21 Liver perfusion studies suggested that triglyceride is more available for secretion in the absence of hepatic SOAT2, resulting in a higher rate of mobilization of hepatic triglyceride.16,19 Interestingly, SOAT2SI−/SI−LDLr−/− mice appeared to have even higher plasma triglyceride than liver-specific SOAT2 knockouts. Absence of intestinal SOAT2 would generate the CE-poor chylomicrons, and remnants delivered to the liver could possibly lead to a similar effect to increase triglyceride mobilization and packaging of more triglyceride into the newly synthesized VLDL and circulating LDL. Furthermore, it is uncertain whether the absence of SOAT2 in the intestine or liver would affect the activities of lipoprotein lipase and its activator apolipoprotein CII, or interaction with apolipoprotein E, which all contribute to apolipoprotein B–containing lipoprotein clearance and atherosclerosis progression. It is important to emphasize that the hypertriglyceridemia in SOAT2 knockout animals does not seem to contribute significantly to the development of atherosclerosis as all of the SOAT2 knockouts have elevated circulating triglyceride yet delayed atherosclerosis progression. The aforementioned mechanisms would warrant future studies, such as liver perfusion assay, for clarification. When we looked at gene expression differences (Online Table I), a 10-fold higher level of SC5-1 mRNA was detected in the intestine of the SOAT2SI−/SI− mice, suggesting that this effect is related to the hypertriglyceridemia of these animals although the mechanism for such an outcome is not clear.

In conclusion, our data clearly demonstrate that both hepatic and intestinal SOAT2-derived CEs can promote the potentially atherogenic CE accumulation that occurs during atherosclerosis progression. The data suggest that SOAT2 in either liver or intestine could possibly be a pharmaceutical target for treatment of atherogenesis.

Sources of Funding
This work was supported by grants from the National Institute of Health (P01-HL49373 to L.L. Rudel) and the American Heart Association (Postdoctoral Fellowship 12POST11070006 to J. Zhang).

Disclosures
None.

References
Novelty and Significance

What Is Known?

- Sterol O-acyltransferase 2 (SOAT2)-derived cholesterol esters (CE) contribute significantly to cholesterol homeostasis.
- The importance of SOAT2-derived CE in atherosclerosis has been apparent from the limited amount of atherosclerosis that develops in whole body SOAT2 knockout mice.

What New Information Does This Article Contribute?

- Conditional knockout of hepatic and intestinal SOAT2 showed that CE derived from both tissues significantly contribute to the progression of atherosclerosis.
- Liver-specific and whole body SOAT2 knockout animals are more protected from atherosclerosis progression than intestine-specific SOAT2 knockouts.
- Cholesterol oleate, the CE product from SOAT2, is a potential marker to predict the severity of atherosclerosis development.

SOAT2 is a microsomal enzyme that converts free cholesterol into CE, many of which get packaged into apolipoprotein B–containing lipoproteins mainly in the liver and intestine. Whole body depletion of SOAT2 in mice has consistently been associated with reduced atherosclerosis. We generated liver-specific and intestine-specific SOAT2 knockout mice to isolate the contribution in plasma of liver- and intestine-derived CE in atherosclerosis development. The absence of SOAT2 in either the liver or small intestine resulted in a retarded atherosclerosis progression. However, 2 separate measurements of atherosclerosis, namely aortic lesion area and aortic CE concentration, were significantly lower in liver-specific SOAT2 knockouts than in intestinal SOAT2 knockouts. Significant positive associations between atherosclerosis extent and plasma low-density lipoprotein (LDL) cholesterol concentration and LDL percentage of cholesterol oleate (mostly from SOAT2) were found for both intestine and liver SOAT2 knockouts, but higher percentages of cholesterol oleate in LDL and more atherosclerosis were found in intestine-specific knock out mice. The correlation with atherosclerosis of LDL percentage cholesterol oleate was stronger than the correlation of atherosclerosis of LDL percentage cholesterol oleate.


Cholesterol Esters (CE) Derived From Hepatic Sterol O-Acyltransferase 2 (SOAT2) Are Associated With More Atherosclerosis Than CE From Intestinal SOAT2

Jun Zhang, Janet K. Sawyer, Stephanie M. Marshall, Kathryn L. Kelley, Matthew A. Davis, Martha D. Wilson, J. Mark Brown and Lawrence L. Rudel

Circ Res. 2014;115:826-833; originally published online September 19, 2014;
doi: 10.1161/CIRCRESAHA.115.304378

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/115/10/826

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2014/09/19/CIRCRESAHA.115.304378.DC1
Online Supplement

Supplemental Methods

**Mouse model**

Generation of tissue-specific SOAT2 knockouts has been described in detail previously\(^1\). Floxed mice (LoxP sites flanked exons 11 through 13 of the SOAT2 gene on chromosome 15) are designated as SOAT2\(^{fl/fl}\). After introducing Cre recombinase driven either by the albumin or villin promoter, SOAT2 was specifically deleted in the liver (SOAT2\(^{fl/fl}\)Alb\(^{Cre+}\)) or small intestine (SOAT2\(^{fl/fl}\)Vil\(^{Cre+}\)), which are designated as SOAT2\(^{L/L-}\) or SOAT2\(^{SI/SI-}\). SOAT2\(^{fl/fl}\) mice were maintained on a mixed background (strains of C57BL/6, 129S6, 129SvEv). To create the conditional knockouts on LDLr\(^{-/-}\) background, SOAT2\(^{L/L-}\) and SOAT2\(^{SI/SI-}\) mice were bred with SOAT2\(^{+/+}\)LDLr\(^{-/-}\) (strain of C57BL/6) or SOAT2\(^{-/-}\)LDLr\(^{-/-}\) (strain of C57BL/6). Genotypes of litters were screened by PCR. Animals used in the study were from breeders set up as follows: heterozygotes of SOAT2\(^{fl/+}\)LDLr\(^{-/-}\) with or without Alb\(^{Cre}\) (or Vil\(^{Cre}\)), SOAT2\(^{fl/-}\)LDLr\(^{-/-}\) with or without Alb\(^{Cre}\) (or Vil\(^{Cre}\)). Essentially equal numbers of both male and female mice were included in the study. At the age of 8 to 9 weeks, the experiments were started by feeding the mice a semisynthetic diet containing 20% of energy as lard with added cholesterol (0.1% wt/wt) for a total of 16 weeks.

**Plasma lipoprotein analyses**

Cholesterol concentrations were measured using a colorimetric enzymatic assay as previously described \(^2\)\(^-\)\(^4\). For LDL isolation, an aliquot of plasma containing about twenty \(\mu\)g of total plasma cholesterol was diluted in phosphate buffered saline into a final volume of 400 \(\mu\)L. After centrifugation to remove any protein precipitates, samples were injected onto a Superose 6 HR 10/30 chromatography column (Amersham Pharmacia), which was subsequently run at 0.4 mL/min with PBS. The signal was obtained as cholesterol using the enzymatic assay of the effluent, which was continuously monitored spectrophotometrically using Chrom Perfect Spirit Software (Justice Laboratory Software). LDL was collected for each plasma sample. Total lipid was extracted with chloroform/methanol (2:1) and phases were split with \(\text{H}_2\text{O}\). The chloroform phase was recovered and dried down under nitrogen and then dissolved in 1 mL chloroform/methanol (1:1). Fifty \(\mu\)L of LDL lipid extract was diluted in 500 \(\mu\)L methanol containing 500 pg/\(\mu\)L of cholesterol heptadecanoate (Nu-Chek Prep) as an internal standard and 1 ng/\(\mu\)L of sodium formate. After standing for 30 min, the solution was analyzed by direct infusion into a Waters Quattro II tandem mass spectrometer operated at a flow rate of 10 \(\mu\)L/min in the positive ion mode. Cholesterol ester species were quantified with a response curve against 0.78 \(\mu\)M internal standard as described elsewhere\(^5\).

**Biliary lipid analysis**

Gallbladder bile was collected at necropsy from fasted mice. Biliary lipids were assayed enzymatically as previously described\(^2\)\(^-\)\(^4\). For analysis of biliary lipid concentrations, a measured volume (5 to 10 \(\mu\)L) of bile was placed into a glass tube and the lipids were extracted in chloroform/methanol (2:1). An aliquot of the chloroform phase was used for enzymatic quantification of cholesterol and phospholipids in a similar fashion to that described for liver lipid measurement. An aliquot of the aqueous phase of the extraction was analyzed for total bile acid content using a hydroxysteroid dehydrogenase-based enzymatic assay.

**Quantification of cholesterol in aortae**

Lipids of the fixed entire aorta were extracted into chloroform/methanol (2:1) overnight after the addition of 20 \(\mu\)g of 5-alpha cholestane as an internal standard\(^2\). Aortic protein was then washed twice with chloroform/methanol (2:1) and pooled solvent containing lipids was evaporated under
nitrogen. Dried lipids were dissolved in 250 µL hexane and 1 µL of hexane phase was injected onto ZB-50 GLC column to measure free cholesterol (FC). After the FC quantification, the remaining samples were saponified and total cholesterol (TC) of aortas was then determined by GLC. Aortic CE was calculated using the equation (TC – FC) × 1.67.

**Real-time PCR analysis of intestinal and hepatic mRNA levels**

Total RNA was extracted from ~100 mg of liver and proximal small intestine with Trizol (Invitrogen Life Technologies) using the protocol provided by the manufacturer. The RNA was resuspended in 300 µl of diethyl pyrocarbonate water and 1 µg of RNA was reverse transcribed to cDNA using qScript reverse transcriptase (Quanta) under the following conditions: 25°C 5 min, 42°C 30 min and 85°C 5min. The cDNA was diluted 1:10 using diethyl pyrocarbonate water and real-time PCR was done in duplicate with 5µl of cDNA, 10 µl of SYBR GREEN PCR mastermix (Roche), 3 µl of diethyl pyrocarbonate water and 1 µl of forward and reverse primer (20 pmol) for a final reaction volume of 20 µl. Primer sequences are as presented in the following Table. PCR was then run on the Sequence Detection System 7500 (Applied Biosystems) using the following conditions: 94°C for 10 min, 94°C 10 sec and 60°C for 1 min. The fluorescence measurement used to calculate threshold cycle (Ct) was made at the 60°C point. A dissociation curve was run at the end of the reaction to ensure a single amplification product. Ct values were entered into the following equation to determine the arbitrary unit value: $1 \times 10^9 \times e^{-0.6931 \times Ct}$. All values were then normalized to cyclophilin mRNA concentration of the sample to take total RNA concentration into account.

<table>
<thead>
<tr>
<th>Primer Table</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyclophilin</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>SCD1</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Srebp 1c</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>DGAT1</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>DGAT2</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>mFAS</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>mACC1</strong></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Supplemental Figures

Online Figure I: HPLC profiles of plasma lipoprotein cholesterol distribution. A representative profile for each experimental group is plotted.
**Online Figure II.** Body weights and liver to body weight ratios for all groups of mice. (A) All mice were fed a semi-synthetic diet containing 0.1% cholesterol for 16wks. Body weight was measured every 4wks. (A) No significant differences among groups in body weights at each time point were present. Liver weights and body weights were measured at necropsy at week16 (B). Data is expressed as ratio of liver weight to body weight. There was no significant difference among genotypes. Data (mean ± SEM) are for 18 to 20 animals per group.
Online Figure III. Total body SOAT2 knockout and intestine-specific, but not liver-specific, deletion of SOAT2 reduces biliary cholesterol levels. A total of 5 to 10μL gall bladder bile were used for the analysis. Lipids were extracted with CHCl₃/MeOH (1:2). Biliary cholesterol (Chol), phospholipids (PL) were measured by enzymatic assay kits. Biliary bile acids (BA) were quantified by enzymatic assay using 3alpha HSD. Values are expressed as percentage molar of individual lipid class to total biliary lipids (sum of Chol, PL and BA). Data represent the mean ± SEM from 16 to 18 mice per group. Bars not sharing common letters differ with $P < 0.05$. ns: not significantly different.
**Online Figure IV.** Relationship between aortic atherosclerosis, measured as CE concentration in aorta and LDL cholesterol concentration in plasma (A) and LDL cholesterol ester composition (B) measured as percentage of cholesterol esters containing monounsaturated fatty acids. Individual experimental groups are indicated with different symbols, as designated in the legend for Figure 5, i.e. filled boxes, SOAT2^{+/+}; filled circles, SOAT2^{fl/fl}; open circles, SOAT2^{-/-}; open triangles, SOAT2^{L-/L-}; open boxes, SOAT2^{S+/-}. The regression coefficient and significance level is shown together with the least squares best fit regression line.
Online Table 1. Liver and intestinal gene expression levels among SOAT2 genotypes of mice

A. Liver

<table>
<thead>
<tr>
<th>Gene</th>
<th>SOAT2 Genotype (AU)</th>
<th>+/+</th>
<th>fl/fl</th>
<th>-/-</th>
<th>L'/L'</th>
<th>S'I/S'I</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCD-1</td>
<td></td>
<td>3.4±0.4</td>
<td>3.9±0.4</td>
<td>3.5±0.4</td>
<td>5.0±0.3</td>
<td>4.4±0.5</td>
</tr>
<tr>
<td>ACC (*1000)</td>
<td></td>
<td>28.3±5.1</td>
<td>32.0±3.4</td>
<td>30.1±1.7</td>
<td>36.9±3.3</td>
<td>37.3±2.9</td>
</tr>
<tr>
<td>FAS (*100)</td>
<td></td>
<td>9.6±3.7</td>
<td>12.5±2.6</td>
<td>7.4±1.2</td>
<td>15.5±2.4</td>
<td>17.1±1.9</td>
</tr>
<tr>
<td>SREBP-1c (*1000)</td>
<td></td>
<td>10.9±0.9</td>
<td>11.4±0.8</td>
<td>11.2±0.6</td>
<td>11.1±0.7</td>
<td>10.1±0.4</td>
</tr>
<tr>
<td>DGAT1 (*100)</td>
<td></td>
<td>12.7±0.4</td>
<td>10.9±0.6</td>
<td>12.1±0.7</td>
<td>12.6±0.5</td>
<td>11.2±0.5</td>
</tr>
<tr>
<td>DGAT2 (*100)</td>
<td></td>
<td>23.7±0.2</td>
<td>26.7±3.3</td>
<td>30.6±1.9</td>
<td>32.0±3.8</td>
<td>31.5±3.0</td>
</tr>
</tbody>
</table>

B. Small Intestine

<table>
<thead>
<tr>
<th>Gene</th>
<th>SOAT2 Genotype (AU)</th>
<th>+/+</th>
<th>fl/fl</th>
<th>-/-</th>
<th>L'/L'</th>
<th>S'I/S'I</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCD-1</td>
<td></td>
<td>1.9±0.1 a</td>
<td>1.9±0.5 a</td>
<td>22.9±3.9 b</td>
<td>1.3±0.4 a</td>
<td>24.5±2.1 b</td>
</tr>
<tr>
<td>ACC (*1000)</td>
<td></td>
<td>9.6±0.5</td>
<td>9.6±0.4</td>
<td>11.4±0.6</td>
<td>10.6±0.5</td>
<td>11.1±0.7</td>
</tr>
<tr>
<td>FAS (*100)</td>
<td></td>
<td>16.7±1.5</td>
<td>15.2±0.9</td>
<td>15.6±1.0</td>
<td>16.7±0.7</td>
<td>15.5±1.1</td>
</tr>
<tr>
<td>SREBP-1c (*1000)</td>
<td></td>
<td>17.8±0.5</td>
<td>17.6±0.5</td>
<td>18.2±0.9</td>
<td>16.8±0.6</td>
<td>18.2±0.9</td>
</tr>
<tr>
<td>DGAT1 (*100)</td>
<td></td>
<td>36.0±1.5</td>
<td>34.4±1.4</td>
<td>33.0±1.0</td>
<td>36.6±1.4</td>
<td>36.8±0.6</td>
</tr>
<tr>
<td>DGAT2 (*100)</td>
<td></td>
<td>19.8±1.7</td>
<td>18.4±2.5</td>
<td>18.6±2.3</td>
<td>15.8±1.4</td>
<td>18.1±1.0</td>
</tr>
</tbody>
</table>
Online References


