Inhibition of Acyl-Coenzyme A:Cholesterol Acyltransferase 2 (ACAT2) Prevents Dietary Cholesterol-associated Steatosis by Enhancing Hepatic Triglyceride Mobilization

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Acyl-CoA:cholesterol O-acyl transferase 2 (ACAT2) promotes cholesterol absorption by the intestine and the secretion of cholesteryl ester-enriched very low density lipoproteins by the liver. Paradoxically, mice lacking ACAT2 also exhibit mild hypertriglyceridemia. The present study addresses the unexpected role of ACAT2 in regulation of hepatic triglyceride (TG) metabolism. Mouse models of either complete genetic deficiency or pharmacological inhibition of ACAT2 were fed low fat diets containing various amounts of cholesterol to induce hepatic steatosis. Mice genetically lacking ACAT2 in both the intestine and the liver were dramatically protected against hepatic neutral lipid (TG and cholesteryl ester) accumulation, with the greatest differences occurring in situations where dietary cholesterol was elevated. Further studies demonstrated that liver-specific depletion of ACAT2 with antisense oligonucleotides prevents dietary cholesterol-associated hepatic steatosis both in an inbred mouse model of non-alcoholic fatty liver disease (SJL/J) and in a humanized hyperlipidemic mouse model (LDLr−/−, apoB100/100). All mouse models of diminished ACAT2 function showed lowered hepatic triglyceride concentrations and higher plasma triglycerides secondary to increased hepatic secretion of TG into nascent very low density lipoproteins. This work demonstrates that inhibition of hepatic ACAT2 can prevent dietary cholesterol-driven hepatic steatosis in mice. These data provide the first evidence to suggest that ACAT2-specific inhibitors may hold unexpected therapeutic potential to treat both atherosclerosis and non-alcoholic fatty liver disease.

Non-alcoholic fatty liver disease (NAFLD) is characterized by neutral lipid (triglyceride and cholesteryl ester (TG and CE)) accumulation in hepatocytes of patients with no history of chronic alcohol use (1, 2). Lipid droplets consisting of neutral lipids (triglyceride and cholesteryl ester) accumulate within the hepatocyte and can contribute 5–10% or more of the total mass of the liver (3). The causes of the lipid accumulation are not always clear, although a popular hypothesis is that excess free fatty acid availability to the liver in situations where energy excess prevails can lead to the condition (4). NAFLD is often asymptomatic and not regularly detected until progression into diseased fibrotic and/or inflamed non-alcoholic steatohepatitis and cirrhosis has occurred (5, 6). NAFLD is estimated to affect at least 20 and 5% of the general adult and child populations, respectively; it affects greater than 50% of the obese population in both age groups (3, 7). It is expected that as the prevalence of obesity and metabolic syndrome increases, NAFLD-associated diseases will be an increasing healthcare concern (8). Currently, there are no known effective treatments for NAFLD, although it is suggested that major changes in diet and exercise programs to reduce obesity could also diminish the severity and progression of NAFLD into non-alcoholic steatohepatitis and cirrhosis (2, 3, 5–9).

The major tissue cholesterol-esterifying enzyme, acyl-CoA: cholesterol O-acyl transferase 2 (ACAT2 also known as SOAT2, sterol O-acyltransferase) is found within lipoprotein-producing cells such as intestinal enterocytes and hepatocytes (10, 11). It has been previously documented that ACAT2 plays a critical role in the production of atherogenic apoB-containing lipoproteins (12) and that ACAT2-specific inhibitors are extremely effective in preventing murine atherosclerosis (13). In addition to direct alterations in cholesterol metabolism, mice lacking ACAT2 exhibit unexpected hypertriglyceridemia, indicating that either increased production or decreased clearance of VLDL TG is altered in these mice (15). Despite this, ACAT2 deletion is highly atheroprotective (14), and it is reasonable to assume that much of the atheroprotective benefit of limiting ACAT2 can be explained by the diminished packaging of hepatic CE into atherogenic VLDL particles.

Data from the present studies have uncovered an unexpected role for ACAT2 in hepatic TG metabolism. We have found that inhibition of ACAT2 not only has the expected outcome of

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Inhibition of ACAT2 Prevents Hepatic Steatosis

lowering hepatic CE but also limits hepatic triglyceride accumulation in several mouse models of dietary cholesterol-induced NAFLD. Based on the rapidly increasing prevalence of obesity in Western societies, an increase in obesity comorbidities seems likely, particularly including diseases with earlier onset in obesity development such as NAFLD (2). The etiology of development of the NAFLD process is unclear, and currently, the treatment options for NAFLD are few (9). Uncovering clues that could help decipher pathways in the progression of NAFLD could greatly facilitate both the prevention and the treatment of this disease. The present studies in mouse models provide support for the possibility that ACAT2-specific inhibitors could limit both atherosclerosis and NAFLD in humans.

EXPERIMENTAL PROCEDURES

Mice and Diets—Wild type (ACAT2+/+) and ACAT2 knockout (ACAT2−/−) mice were generously provided by Dr. Robert Farese, Jr. (Gladstone Institute, San Francisco, CA) and were maintained on a mixed genetic background (62.5% C57BL/6, 25% 129 SvJae, 12.5% SvEv). Female ACAT2−/− littermates were used as controls in all studies to minimize genetic heterogeneity. At 6 weeks of age, these mice were switched from a diet of rodent chow to a homemade low fat (10% of energy as palm oil) diet containing either 0.001% (w/w) or 0.2% (w/w) cholesterol, and they were maintained on this diet for 6 additional weeks. For antisense oligonucleotide (ASO) studies using inbred mouse strains (C57BL/6, C57L/J, SJL/J, SM/J, SWR/J), male and female mice were purchased from The Jackson Laboratory (Bar Harbor, ME) based on previous comparisons of atherosclerosis and hepatic lipid accumulation susceptibility (16). At 6 weeks of age, the inbred mice were switched from a diet of rodent chow to a homemade low fat (10% of energy as palm oil) diet containing either 0.001% (w/w) or 0.2% (w/w) cholesterol, and they were maintained on this diet for 6 additional weeks. ASO-mediated knockdown in hyperlipidemic low density lipoprotein receptor-deficient (LDLr−/−), apolipoprotein-B-100 only (apoB100/100) mice was carried out as described previously in mice consuming a low fat (20% of energy as palm oil) diet containing 0.1% (w/w) cholesterol for 8 weeks (17). All animals used in these studies were housed in an American Association for Accreditation of Laboratory Animals (AAALAC)-approved animal facility. Protocols for all studies were preapproved by the Wake Forest University Animal Care and Use Committee (ACUC).

ASO Treatment—Phosphorothioate-modified ASOs were obtained from ISIS Pharmaceuticals (Carlsbad, CA), and have been described previously (13, 17). Normallipidemic male SJL/J mice were randomly assigned to groups to receive treatment with either a control non-targeting ASO (control ASO; 5′-TCC-CATTTCAGGAGACCTGG-3′) or an ASO specifically targeting ACAT2 (ACAT2 ASO; 5′-GCTCTAATCACCTCA-GAACT-3′). Biweekly, intraperitoneal injections of 25 mg/kg/week of ASO were started at 6 weeks of age and were continued for 6 weeks in inbred (SJL/J) mice or for 8 weeks in hyperlipidemic (LDLr−/−, apoB100/100) mice.

Hepatic Lipid Measurements—Lipid extraction and biochemical determination of hepatic lipid mass were conducted as described previously (17–19). For histologic evaluation, frozen tissue samples were thawed into neutral buffered formalin for 48 h at 4 °C. Liver samples were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

ACAT2 Activity Assays and Western Blot Analysis—Total liver homogenates (17) and hepatic microsomal membranes (20, 21) were prepared as described previously from snap-frozen livers. Assays for ACAT2 activity were performed as described (17) using 50-μg aliquots of total protein from liver microsomal membrane suspensions. For immunoblotting of ACAT2 in SJL/J mouse livers, microsomes were used as a protein source, and Western blotting was conducted as described previously (17–19).

Quantitative Real Time PCR—RNA extraction and quantitative real time PCR were conducted as described previously (19). Primer pairs used are described in supplemental Table 1.

Plasma Lipid Analyses—Mice were fasted for 4 h prior to blood collection by submandibular bleed at the indicated time points. Detailed descriptions of plasma lipid and lipoprotein analyses have been provided previously (12, 17–19). For blood glucose measurements, mice were fasted for 6 h, and blood glucose was monitored using a commercial glucometer (Ascensia Contour, Bayer).

Isolated Liver Perfusion and Liver Lipid Secretion—Isolated recirculating liver perfusion was performed according to the protocol of Lee et al. (22) to estimate direct secretion rates of lipids in nascent apoB-containing lipoproteins.

Isotope Labeling of Hepatic and Secreted Lipids—Approximately 1 h prior to surgery, non-fasted mice were injected with an intraperitoneal 200-μl bolus containing 20 μCi of [3H]oleic acid in 5% bovine serum albumin with 0.75% cold oleic acid as a carrier for delivery of 3H-fatty acid to the liver (23). About 8% of the injected dose was found to be incorporated into liver lipids at 1 h after injection, and >85% of this was in triglycerides. Mice were returned to their cage until their perfusion surgery start time 1 h later, at which point they were anesthetized with isoflurane. After surgery and successful cannulation of the circulation into and out of the liver, perfusion at 1 ml/min with recirculating medium was initiated (22), and a constant infusion of [14C]oleic acid complexed to albumin was pumped into the perfusion medium at a fixed rate of ~11,000 dpm per minute. Perfusate aliquots (1.5 ml at each time point) were taken at half-hour intervals, and fresh medium was added back to maintain a constant medium volume of 10 ml. The appearance rates of [3H]oleic acid and [14C]oleic acid in perfusate triglyceride were monitored as a function of hepatic triglyceride mobilization ([3H]oleate) versus new triglyceride synthesis ([14C]oleate).

Liver biopsies were taken at 1- and 2-h time points, and a sample was collected at the end of perfusion at 3 h. During surgical isolation of the liver, 4.0 suture silks were looped around two separate liver lobes and secured. At 1 h, one of the loops was tightened around the liver lobe (~0.1 g) to constrict blood flow into the lobe, which was then removed with a pair of surgical scissors, and surgical glue was immediately applied to the cut site to prevent leakage. At 2 h of perfusion, a second lobe was isolated and removed. The timed liver biopsies were immediately weighed and snap-frozen in liquid nitrogen for later analyses to determine the extent of triglyceride labeling with [14C]oleate to estimate hepatic triglyceride synthesis rate during the experiment. The rate of TG mobilization was calculated.
from the $[^{3}H]$oleate appearance in perfusate TG, whereas the rate of new synthesis and deposition was calculated from the $[^{14}C]$oleate kinetics of appearance in hepatic TG. At the completion of the 3-h experiment, all perfusion medium was collected, and the liver was weighed and snap-frozen in liquid nitrogen. Liver and perfusate lipids were extracted and analyzed as above. Recovery of isotope-labeled lipids was determined after separation of lipid extracts on thin layer chromatography (TLC) plates using a solvent system of hexane:ethyl min. Liver and perfusate lipids were extracted and analyzed, and the liver was weighed and snap-frozen in liquid nitrogen. Liver and perfusate lipids were extracted and analyzed as above. Recovery of isotope-labeled lipids was determined after separation of lipid extracts on thin layer chromatography (TLC) plates using a solvent system of hexane:ethyl min.

RESULTS

In Addition to Lowering Hepatic Cholesteryl Ester Accumulation, Disruption of ACAT2 Lowers Hepatic Triglyceride Accumulation while Raising Plasma Triglyceride in a Dietary Cholesterol-dependent Manner—ACAT2 is the main cholesterol-esterifying enzyme in the liver and intestine, and genetic deletion of this enzyme in mice has been shown to lower both intestinal cholesterol absorption and hepatic cholesteryl ester accumulation when mice are challenged with cholesterol in the diet (15, 21, 22). As seen in previous studies (17, 19), 6 weeks of feeding low cholesterol (0.001% w/w) or high cholesterol (0.2% w/w) diets resulted in lower hepatic free cholesterol in ACAT2$^{-/-}$ mice when compared with ACAT2$^{+/+}$ mice (Fig. 1A). Hepatic CEs were at least 10-fold higher when wild type mice were fed the higher cholesterol diet (Fig. 1B), whereas livers from ACAT2-deficient mice (ACAT2$^{-/-}$) and mice treated with ACAT2 ASO were protected from accumulation of CE when either diet was fed. The levels of hepatic triglyceride closely followed the cholesteryl ester levels so that the ACAT2$^{+/+}$ mice fed the 0.2% cholesterol diet had significantly higher hepatic triglyceride concentrations (Fig. 1C) as well as elevated CE concentrations. Intriguingly, plasma triglyceride concentrations were inversely affected and consistently were lower when hepatic triglycerides were higher (Fig. 1D).

Consistent with the biochemistry of liver lipids, after hematoxylin and eosin staining, histological analysis of ACAT2$^{+/+}$ mouse livers showed several large lipid droplets within most hepatocytes, typical of NAFLD, whereas ACAT2$^{-/-}$ livers had fewer and smaller lipid droplets (Fig. 2). To make sure that this outcome was not an unusual consequence of the mixed strain background of these mice, male and female mice of several inbred strains were purchased from The Jackson Laboratory based on previous studies on the susceptibility among these strains of mice to develop liver steatosis (16). After 6 weeks of feeding the high cholesterol diets (0.2% w/w cholesterol), mice were fasted for 4 h, livers were collected, and lipid concentrations were determined. Hepatic triglyceride was highest in the SJL/J male mice and lowest in the C57L/J male mice (Fig. 3A), with all other strains and genders having intermediate values. ACAT2 was not disrupted in any of these inbred strains of mice so that hepatic cholesteryl ester was present in all livers (data not shown). SJL/J male mice were selected for further studies with an ASO targeted to ACAT2 to see whether depletion of hepatic ACAT2 activity would alter the cholesteryl ester as well as the triglyceride response in the liver of SJL mice.

Knockdown of ACAT2 with ASO Provides Protection from Dietary Cholesterol-dependent Hepatic Triglyceride Accumulation—Treatment of male SJL/J mice with an ACAT2-targeted ASO lowered hepatic ACAT2 mRNA (data not shown), protein (Fig. 3B), and ACAT2 activity (Fig. 3C) when compared with treatment with a non-targeting control ASO. Targeted knockdown of ACAT2 in SJL mice lowered hepatic triglyceride accumulation (Fig. 3D) and hepatic cholesteryl ester accumulation (Fig. 3E) (as was the case for the ACAT2 ASO-treated hyperlipidemic mice of Fig. 1), without altering hepatic phospholipid concentration (Fig. 3F).

Previous studies have shown that ASO-mediated knockdown of ACAT2 can lower hepatic cholesteryl ester when compared with control ASO-treated mice on a hyperlipidemic (apoB$^{100/100}$,LDLr$^{-/-}$) background (11, 12). In the data shown here, responses in livers from ASO-treated apoB$^{100/100}$,
LDLR−/− mice fed a 0.1% (w/w) cholesterol diet for 8 weeks (Fig. 1) were consistent with the effect in normolipidemic SJL mice (Fig. 3) so that knockdown of ACAT2 in mice of either background resulted in lower hepatic triglyceride concentrations when compared with their ASO-treated controls. At the same time, consistent with previous studies and the expected role of ACAT2, ASO-mediated knockdown of ACAT2 also significantly lowered hepatic cholesteryl ester (Figs. 1B and 3E).

Consistency in the Association between Hepatic CE and Triglyceride Concentrations—Our data suggest that ACAT2 depletion is consistently associated with a major decrease in hepatic cholesteryl ester and also with a decrease in liver triglycerides. Values of hepatic cholesteryl ester concentrations in a variety of experimental mice with and without ACAT2 fed different dietary cholesterol levels were plotted against hepatic triglyceride concentrations (Fig. 4), and a strongly significant and positive association with a correlation coefficient of \( r = 0.87 \) (\( p < 0.0001 \)) was defined by these data. This finding suggests that the two hepatic lipids are metabolically linked across a wide range of concentrations through a mechanism that remains to be defined. Because ACAT2 KO leads to lower triglycerides and dietary cholesterol leads to higher hepatic triglycerides, we...
made the assumption that cholesteryl ester levels are of key importance.

**Nascent Lipoprotein Secretion Rate during Liver Perfusion Depends on ACAT2 Status**—Liver perfusion experiments were done to examine possible mechanisms for the neutral lipid association. During isolated liver perfusion, the livers from mice deficient in ACAT2 secreted lipoprotein particles with a neutral lipid core that was essentially only triglyceride (Fig. 5A) and depleted in cholesteryl ester (Fig. 5B). Secretion of free cholesterol was not significantly different (Fig. 5C). Further, the diameter (~85 nm) of the isolated perfusate VLDL did not differ based on ACAT2 status, as determined using a dynamic light scatter instrument (Fig. 5D). In other work, these differences were reproduced in a second set of liver perfusion experiments where pure strain C57Bl6 mice with and without ACAT2 were studied (data not shown). Thus, the data in two separate sets of perfusion experiments (using mixed strain mice (Fig. 5) and pure C57Bl6/J mice) demonstrated that an important effect of ACAT2 deletion is to promote a significant increase in VLDL triglyceride secretion in the form of increased numbers of VLDL particles of similar size to those secreted by livers from ACAT2+/+ mice.

**Examination of Gene Expression in the Regulation of ACAT2-related Modifications of Hepatic Lipid Metabolism**—Mice with a disruption in ACAT2 can become hypertriglyceridemic and have livers that are depleted in cholesteryl ester and reduced in triglyceride concentration but still secrete triglyceride at a higher rate than livers from ACAT2 intact animals. Accordingly, we looked for changes in hepatic gene expression that could control such modifications in liver lipid metabolism. Expression of genes was examined using reverse transcription-PCR. We monitored several groups of genes whose protein products are involved in (a) lipogenesis, including sterol regulatory element-binding protein-1c (SREBP-1c), fatty acid synthase (FAS), stearoyl-CoA desaturase-1 (SCD-1), acetyl-CoA carboxylase-1 (ACC-1), and tribbles-3 (TRB3); (b) triglyceride synthesis and degradation, including diacylglycerol acyltransferase 1 and 2 (DGAT1 and DGAT2), triacylglycerol hydrolase 1 and 2 (TGH1 and TGH2), adipose tissue triacylglycerol lipase (ATGL), comparative gene identification-58 (CGI58), also termed ADHD5, carnitine palmitoyltransferase 1 (CPT1), and mitochondrial glycerol phosphate acyltransferase 1 (mGPAT1); (c) hepatic inflammation including interleukin 6 (IL6) and cluster of differentiation-68 (CD68); and (d) miscellaneous aspects of lipid metabolism, including ATP binding cassette transporter G5 (ABCG5), 3-hydroxy-3-methylglu-
Inhibition of ACAT2 Prevents Hepatic Steatosis

Acyl-CoA thioesterase (HMGCOSA Syn), adipose differentiation-related protein (ADRP), cell death-inducing DNA fragmentation factor α (DFFA)-like effector B (CIDE-B), microsomal triglyceride transfer protein (MTP), fibroblast growth factor 21 (FGF21), fibroblast growth factor receptor 4 (FGFR4), cytochrome P-450 3A11b (CYP3A11B), small heterodimeric partner (SHP), and Lipin1. Data from two separate experiments were compared. Experiment 1 measured the mRNA abundance of genes in livers of normolipidemic ACAT2+/+ and ACAT2−/− mice, whereas the second experiment was done in mice treated with a control or ACAT2 ASO. In both cases, no significant transcriptional changes were identified so that the mechanism(s) for the differences in hepatic lipid accumulation between ACAT2+/+ and ACAT2−/− mice remains unexplained. Statistically significant decreases (>95%) were measured in the expression of ACAT2 in both experiments to confirm that where present, significant differences would be detected. However, no significant differences in gene expression were identified to account for the shift in the hepatic triglyceride accumulation, mobilization, and secretion that was induced by ACAT2 disruption.

Fasting blood glucose was also monitored in subsets of ACAT2+/+ and ACAT2−/− mice to determine whether these mice had altered glucose metabolism that often accompanies hepatic steatosis. However, there were no differences in fasting blood glucose between ACAT2+/+ and ACAT2−/− animals at baseline or after 10 or 20 weeks of high dietary cholesterol feeding. Therefore, the diet-induced NAFLD described here is not apparently associated with insulin resistance and hyperglycemia secondary to steatosis.

Measurement of Rates of Hepatic Triglyceride Synthesis and Mobilization by Liver Perfusion—In the absence of data suggesting that ACAT2 exerts major effects on gene expression of enzymes involved in synthesis of hepatic lipids, we designed experiments to test the hypothesis that the presence of cholesteryl ester in the lipid droplet could reduce the rate of triglyceride mobilization assuming that hydrolysis and resynthesis of triglyceride in the lipid droplet are required for its subsequent secretion in VLDL. For these experiments, hepatic lipids were prelabeled 1 h before perfusion with [3H]oleate so that the rate of mobilization of preformed triglyceride could be monitored during liver perfusion. Further, isolated liver perfusion was done with a continuous infusion of [14]Coleate to permit estimation of the rates of nascent hepatic triglyceride synthesis to be estimated. In comparing the apparent secretion rates between the livers of ACAT2+/+ and ACAT2−/− mice, a higher rate of appearance in perfusate for triglyceride mass (Fig. 5A, for [3H]oleate-labeled TG (Fig. 6C) and for [14]Coleate-labeled triglyceride (Fig. 6A)) was seen for ACAT2−/− livers. Further, when monitoring accumulation rates of

DISCUSSION

Dietary cholesterol has long been recognized to play a role in the development of cardiovascular disease (25). The findings presented here demonstrate an association between dietary cholesterol and the development of NAFLD in relevant mouse models. When absorbed cholesterol enters the body, it is esterified by ACAT2 and directed to the liver, where some of it can be stored within hepatocytes in lipid droplets as CEs (26). Data presented here show a direct correlation between the concentration of hepatic CE (adjusted upwards by diet or downward by limiting ACAT2 expression) and TG. We have demonstrated that when excess stored CE molecules are present in the liver, the mobilization of hepatic TG is limited and TG secretion is reduced, both resulting in retention of neutral lipids (CE and TG) within the liver in lipid droplets, as is typical of hepatic steatosis. The sequence of events leading from NAFLD to non-alcoholic steatohepatitis and onto cirrhosis is thought to be important in the development of liver disease. In this context, it is important to note that in a study of 9221 participants with 13.3 years of follow-up, 118 diagnoses of cirrhosis and 5 diagnoses of liver cancer were positively linked to consumption of higher levels of dietary cholesterol (27). Clearly, the role of dietary cholesterol, with the subsequent increased hepatic esteri-
Inhibition of ACAT2 Prevents Hepatic Steatosis

JOURNAL OF BIOLOGICAL CHEMISTRY

5 R. Temel and L. L. Rudel, unpublished findings.
data not shown, homogenates were made from the frozen liver samples of ASO-treated hyperlipidemic mice, as described (12). 25 μg of total protein were loaded per well, and immunoblots were performed to compare the protein expression of SCD1, ACC1, and p-AMPK. There was a trend toward lower expression of SCD1 from livers that had ACAT2 knocked down, but there was no change in protein expression between ASO control and ACAT2 ASO-treated livers for ACC1 or p-AMPK. Taken together, these data provide little convincing evidence that there is a general decrease in the expression of genes involved in the de novo synthesis of hepatic TG that could lead to decreased hepatic triglyceride concentrations of the ACAT2-depleted mouse livers.

In summary, the current studies have implicated a novel mechanism by which the accumulation of hepatic cholesteryl ester can lead to hepatic triglyceride accumulation. The mechanism by which this occurs is apparently not through an enhancement in lipogenesis and de novo triglyceride synthesis coupled to insulin resistance and hyperglycemia as is often reported in mice fed high fat diets (34, 35). Instead, the current data indicate that the presence of cholesteryl ester is able to limit the mobilization of triglycerides from the liver, perhaps through the relative substrate flexibility previously reported in some neutral lipid-esterifying enzymes (32, 33), such that the presence of CE interferes with TG hydrolysis and mobilization from cytoplasmic lipid droplets independent of lipogenesis. This study provides novel insight into dietary cholesterol-driven NAFLD and provides additional evidence that ACAT2-specific inhibitors hold promise to not only protect against atherosclerosis but also NAFLD.

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REFERENCES
1. Ludwig, J., Viggiano, T. R., McGill, D. B., and Oh, B. J. (1980) Mayo Clin. Proc. 55, 434–438
2. Schreuder, T. C., Verwer, B. J., van Nieuwkerk, C. M., and Mulder, C. J. (2008) World J. Gastroenterol. 14, 2474–2486
3. Varela-Rey, M., Embade, N., Ariz, U., Lu, S. C., Mato, J. M., and Martínez-Chantar, M. L. (2009) Int. J. Biochem. Cell Mol. Life Sci. 41, 969–976
4. Bradbury, M. W. (2006) Am. J. Physiol. Gastrointest. Liver Physiol. 290, G194-G198
5. Adams, L. A., and Angulo, P. (2006) Postgrad. Med. J. 82, 315–322
6. Marra, F., Gastaldelli, A., Svegliati Baroni, G., Tell, G., and Tirielli, C. (2008) Trends. Mol. Med. 14, 72–81
7. Wieczowska, A., McCullough, A. J., and Feldstein, A. E. (2007) Hepatology 46, 582–589
8. Fan, J. G. (2008) J. Dig. Dis. 9, 63–67
9. Adams, L. A., Lymp, J. F., St Sauver, J., Sanderson, S. O., Lindor, K. D., Feldstein, A., and Angulo, P. (2005) Gastroenterology 129, 113–121
10. Lee, R. G., Willingham, M. C., Davis, M. A., Skinner, K. A., and Rudel, L. L. (2000) J. Lipid Res. 41, 1991–2001
11. Parini, P., Davis, M., Lada, A. T., Erickson, S. K., Wright, T. L., Gustafsson, U., Sahlin, S., Einarsson, C., Eriksson, M., Angelin, B., Tomoda, H., Omura, S., Willingham, M. C., and Rudel, L. L. (2004) Circulation 110, 2017–2023
12. Lee, R. G., Kelley, K. L., Sawyer, J. K., Farese, R. V., Jr., Parks, J. S., and Rudel, L. L. (2004) Circ. Res. 95, 998–1004
13. Bell, T. A., 3rd, Brown, J. M., Graham, M. J., Leonidou, K. M., Crooke, R. M., and Rudel, L. L. (2006) Arterioscler. Thromb. Vasc. Biol. 26, 1814–1820
14. Bell, T. A., 3rd, Kelley, K., Wilson, M. D., Sawyer, J. K., and Rudel, L. L. (2007) Arterioscler. Thromb. Vasc. Biol. 27, 1396–1402
15. Willner, E. L., Tow, B., Buhman, K. K., Wilson, M., Sanan, D. A., Rudel, L. L., and Farese, R. V., Jr. (2003) Proc. Natl. Acad. Sci. U.S.A. 100, 1262–1267
16. Nishina, P. M., Wang, J., Toyofuku, W., Kuypers, F. A., Ishida, B. Y., and Paigen, B. (1993) Lipids 28, 599–605
17. Brown, J. M., Bell, T. A., 3rd, Alger, H. M., Sawyer, J. K., Smith, T. L., Kelley, K., Shah, R., Wilson, M. D., Davis, M. A., Lee, R. G., Graham, M. J., Crooke, R. M., and Rudel, L. L. (2008) J. Biol. Chem. 283, 10522–10534
18. Brown, J. M., Chung, S., Sawyer, J. K., Degirolamo, C., Alger, H. M., Nguyen, T. H., Zhu, X., Duong, M. N., Wibley, A. L., Shah, R. M., Davis, M. A., Kelley, K., Wilson, M. D., Kent, C., Parks, J. S., and Rudel, L. L. (2008) Circulation 118, 1467–1475
19. Temel, R. E., Lee, R. G., Kelley, K. L., Davis, M. A., Shah, R., Sawyer, J. K., Wilson, M. D., and Rudel, L. L. (2005) J. Lipid Res. 46, 2423–2431
20. Carr, T. P., Parks, J. S., and Rudel, L. L. (1992) Arterioscler. Thromb. 12, 1274–1283
21. Rudel, L. L., Davis, M., Sawyer, J. K., Farese, R. V., Jr., Parks, J. S., and Rudel, L. L. (2002) J. Biol. Chem. 277, 31401–31406
22. Lee, R. G., Shah, R., Sawyer, J. K., Hamilton, R. L., Parks, J. S., and Rudel, L. L. (2005) J. Lipid Res. 46, 1205–1212
23. Van Harken, D. R., Dixon, C. W., and Heimberg, M. (1969) J. Biol. Chem. 244, 2278–2285
24. Demel, R. A., and Jackson, R. L. (1985) J. Biol. Chem. 260, 9589–9592
25. McGill, H. C., Jr. (1979) Am. J. Clin. Nutr. 32, 2664–2702
26. Wilson, M. D., and Rudel, L. L. (1994) J. Lipid Res. 35, 943–955
27. Ioannou, G. N., Morrow, O. B., Connole, M. L., and Lee, S. P. (2009) Hepatology 50, 175–184
28. Davis, R. A., McNeal, M. M., and Moses, R. L. (1982) J. Biol. Chem. 257, 2634–2640
29. Anderson, R. A., Joyce, C., Davis, M., Reagan, J. W., Clark, M., Shelnesh, G. S., and Rudel, L. L. (1998) J. Biol. Chem. 273, 26747–26754
30. Cases, S., Novak, S., Zheng, Y. W., Myers, H. M., Lear, S. R., Sande, E., Welch, C. B., Luis, A. I., Spencer, T. A., Krause, B. R., Erickson, S. K., and Farese, R. V., Jr. (1998) J. Biol. Chem. 273, 26755–26764
31. Alam, M., Gilham, D., Vance, D. E., and Lehrer, R. (2006) J. Lipid Res. 47, 375–383
32. Dolinsky, V. W., Gilham, D., Alam, M., Vance, D. E., and Lehrer, R. (2004) Cell Mol. Life Sci. 61, 1633–1651
33. Ghosh, S., Mallon, D. H., Hylemon, P. B., and Grogan, W. M. (1995) Biochim. Biophys. Acta 1259, 305–312
34. Chantar, M. L. (2009) Trends. Mol. Med. 14, 147–152
35. Browning, J. D., and Horton, J. D. (2004) J. Clin. Invest. 114, 147–152