Published in final edited form as:

Curr Opin Lipidol. 2006 February ; 17(1): 85–88.

Macrophage ACAT depletion: mechanisms of atherogenesis

David Akopian and Jheem D. Medh

Department of Chemistry and Biochemistry, California State University at Northridge, Northridge, California, USA

Cellular cholesterol exists both as a free sterol and as esterified cholesterol. Acyl-coenzyme A:cholesterol acyltransferase (ACAT) catalyzes the esterification of excess cellular cholesterol with fatty acids [1]. Two ACAT isoforms have been identified in humans: ACAT1 and ACAT2, each with different expression patterns and unique physiological functions. ACAT2 is expressed exclusively in the liver and intestine, where it participates in lipoprotein assembly. ACAT1 is present in most tissues, and is the primary ACAT isoform in macrophages and steroidogenic tissues. ACAT1 expression is up-regulated during differentiation of monocytes to macrophages and it is abundantly expressed in macrophage foam cells of atherosclerotic lesions.

ACAT plays a major role in cholesterol homeostasis. In the liver and intestine, ACAT2 esterifies newly synthesized or dietary cholesterol prior to incorporation into lipoproteins and thereby regulates the levels of plasma cholesterol. In cells, including macrophages, ACAT regulates the distribution of cellular cholesterol into free and esterified-cholesterol pools. Esterification of cholesterol results in its storage in cellular lipid droplets, making it unavailable for ABCA1-mediated efflux from the cell and leading to the formation of macrophage-foam cells.

Early studies attempted to lower both plasma and macrophage cholesterol content by pharmacological inhibition of ACAT activity [2•]. In experimental animals, non-selective ACAT inhibitors reduced atherosclerosis with or without a parallel decrease in plasma cholesterol levels. Opposite results were obtained, however, in studies of *ACAT1* gene deletion in mouse models of hypercholesterolemia and atherosclerosis. In LDL receptor or apoE knock-out mice, selective deficiency of the ACAT1 isoform resulted in larger atherosclerotic lesions, despite lower plasma cholesterol levels [3]. Also, the lesions were rich in free cholesterol but poor in macrophages [4]. The detrimental effects of *ACAT1* deficiency were attributed to increased macrophage apoptosis due to massive accumulation of free cholesterol [5]. Two significant differences may account for the contradictory results obtained with pharmacological inhibition compared with genetic deficiency of ACAT. ACAT inhibitors reduce the activity of both ACAT isoforms and therefore probably alter the composition of chylomicrons/VLDL particles, making them less atherogenic. Also, pharmacological inhibition is not complete, thus allowing an up-regulated cholesterol efflux pathway to counter moderate accumulation of cellular-free cholesterol.

The controversy over the contribution of ACAT to atherosclerosis was recently renewed, with several publications further characterizing the effect of *ACAT1* deficiency on different facets of the atherosclerotic process. These studies confirm that ACAT depletion in macrophages is pro-atherogenic and shed some light on the underlying biochemical mechanisms. Macrophage-

specific deletion of *ACAT1* resulted in increased atherosclerosis and apoptosis in the proximal aorta of apoE-null mice [6••]. To get around the argument that ACAT deficiency is detrimental due to cytotoxicity of accumulated free cholesterol, Dove *et al.* [7••] used mild cholesterol-loading conditions and demonstrated that cellular cholesterol efflux was significantly reduced in *ACAT1*^(-/-) mouse peritoneal macrophages relative to wild-type cells. This decrease was independent of cell death. *ACAT1* depletion appeared to alter ABCA1 protein stability, as *ABCA1* mRNA was induced but protein levels were lower compared with *ACAT1*^(+/+) macrophages [6••].

Bone marrow transplantation techniques were used to obtain macrophage *ACAT1* depletion in the presence and absence of apoE [6••]. The absence of *ACAT1* did not alter atherogenesis when macrophage apoE was present. Thus, *ACAT1* depletion exacerbates atherosclerosis in hyperlipidemic mice but does not appear to actually trigger it. The results also reiterate a role for apoE in atheroprotection. Impairment of *ABCA1*-mediated cholesterol efflux in apoE/*ACAT1* double knock-out macrophages, however, was not corrected after restoration of apoE expression [6••]. Thus, a major effect of *ACAT1* depletion may be an impairment of cholesterol efflux, but other factors may contribute to atherogenesis. Consistent with this hypothesis, microarray analysis of *ACAT*^(-/-) macrophages revealed an induction of genes involved in inflammation and apoptosis [6••].

ACAT1 gene deletion also led to an increase in cholesterol biosynthesis [8••]. Down-regulation of cholesterol biosynthesis by cholesterol-loading of cells was relatively inefficient in *ACAT1*^(-/-) macrophages, suggesting an impairment of the cholesterol sensing mechanism. This was reflected by a dramatic increase in sterol regulatory element binding protein 1a (*SREBP1a*) mRNA in *ACAT*^(-/-) macrophages. *ACAT1* depletion also appeared to affect cholesterol trafficking. Macrophages from *ACAT1*^(-/-) mice were rich in intracellular vesicles, which may serve to sequester cholesterol into a pool not available for cholesterol efflux [7••]. These studies point to a general disruption of cellular cholesterol homeostasis that undoubtedly contributes to atherogenesis.

Results from pharmacological inhibition of ACAT continue to support this strategy as atheroprotective [9••,10••]. Treatment of mouse peritoneal macrophages with an ACAT inhibitor significantly reduced 7-ketocholesterol-induced apoptosis [9••]. Similarly, TUNEL assays revealed that macrophages from *ACAT1*^(-/-) mice have lower apoptosis compared with wild-type cells. Kharbanda *et al.* [10••] are the first to report the effects of systemic ACAT inhibition in humans. Hypercholesterolemic human subjects treated for 8 weeks with an ACAT inhibitor showed a small reduction in plasma cholesterol levels, but had significantly lower levels of circulating tumor necrosis factor- α , a proinflammatory cytokine. Treatment with ACAT inhibitor also improved endothelium function in these subjects. As inflammation and endothelial dysfunction are common features of atherosclerosis, ACAT inhibitors may prove to be of significant therapeutic value.

Contrary to macrophages, aortic smooth muscle cells (ASMC) were shown to be resistant to cytotoxicity of cholesterol accumulation and ACAT inhibition [11••]. Cholesterol loading of ASMC resulted in foam cell formation and a marked increase in cellular ACAT activity. Treatment of cholesterol-loaded ASMC with an ACAT inhibitor reduced ACAT activity in a dose-dependent manner to below basal levels and reduced foam cell formation without any cell death. As muscle cells also contribute foam cells to atherosclerotic lesions, it is likely that ASMC regulate the maturation and stability of the atherosclerotic plaque. Pharmacological inhibition of ACAT activity will most likely halt progression of ASMC-rich lesions. As most gene-deletion studies have targeted macrophages, they have failed to reveal any benefits of *ACAT1* depletion. It will be interesting to study the effect of smooth muscle-specific deletion of *ACAT1* gene on atherosclerosis.

Abbreviations

ACAT, acyl-coenzyme A:cholesterol acyltransferase; ASMC, aortic smooth muscle cells.

References

1. Buhman KF, Accad M, Farese RV Jr. Mammalian acyl-CoA:cholesterol acyltransferases. *Biochim Biophys Acta* 2000;1529:142–154. [PubMed: 11111084]
2. Leon C, Hill JS, Wasan KM. Potential role of acyl-coenzyme A:cholesterol transferase (ACAT) inhibitors as hypolipidemic and antiatherosclerosis drugs. *Pharm Res* 2005;22:1578–1588. [PubMed: 16180116]
3. Accad M, Smith SJ, Newland DL, et al. Massive xanthomatosis and altered composition of atherosclerotic lesions in hyperlipidemic mice lacking acyl CoA:cholesterol acyltransferase 1. *J Clin Invest* 2000;105:711–719. [PubMed: 10727439]
4. Fazio S, Major AS, Swift LL, et al. Increased atherosclerosis in LDL receptor-null mice lacking *ACAT1* in macrophages. *J Clin Invest* 2001;107:163–171. [PubMed: 11160132]
5. Warner GJ, Stoudt G, Bamberger M, et al. Cell toxicity induced by inhibition of acyl coenzyme A:cholesterol acyltransferase and accumulation of unesterified cholesterol. *J Biol Chem* 1995;270:5772–5778. [PubMed: 7890706]
6. Su YR, Dove DE, Major AS, et al. Reduced *ABCA1*-mediated cholesterol efflux and accelerated atherosclerosis in apolipoprotein E-deficient mice lacking macrophage-derived *ACAT1*. *Circulation* 2005;111:2373–2381. [PubMed: 15851589]
7. Dove DE, Su YR, Zhang W, et al. *ACAT1* deficiency disrupts cholesterol efflux and alters cellular morphology in macrophages. *Arterioscler Thromb Vasc Biol* 2005;25:128–134. [PubMed: 15499044]
8. Dove DE, Su YR, Swift LL, et al. *ACAT1* deficiency increases cholesterol synthesis in mouse peritoneal macrophages. *Atherosclerosis*. 2005 Sept 3; PMID: 16144700 [Epub ahead of print]
9. Freeman NE, Rusinol AE, Linton M, et al. Acyl-coenzyme A:cholesterol acyltransferase promotes oxidized LDL/oxysterol-induced apoptosis in macrophages. *J Lipid Res* 2005;46:1933–1943. [PubMed: 15995174]
10. Kharbanda RK, Wallace S, Walton B, et al. Systemic acyl-CoA:cholesterol acyltransferase inhibition reduces inflammation and improves vascular function in hypercholesterolemia. *Circulation* 2005;111:804–807. [PubMed: 15699266]
11. Rong JX, Kusunoki J, Oelkers P, et al. Acyl-coenzyme A (CoA):cholesterol acyltransferase inhibition in rat and human aortic smooth muscle cells is nontoxic and retards foam cell formation. *Arterioscler Thromb Vasc Biol* 2005;25:122–127. [PubMed: 15499046]

Recommended reading

12. Accad M, Smith SJ, Newland DL, et al. RV: massive xanthomatosis and altered composition of atherosclerotic lesions in hyperlipidemic mice lacking acyl CoA:cholesterol acyltransferase 1. *J Clin Invest* 2000;105:711–719. [PubMed: 10727439] Two mouse models of atherosclerosis were obtained by crossing ACAT-deficient mice with the mice lacking either Apo E or LDLR, and the effect of ACAT deficiency on atherosclerosis was examined. In both models, ACAT deficiency did not decrease atherosclerotic lesions; rather, it resulted in major accumulation of cholesterol deposits in the skin and the brain. The lesions, however, were qualitatively different in ACAT-deficient mice, with reduced cholesteryl ester and macrophage content, presumably due to cytotoxicity of free cholesterol. Bone marrow transplantation of ACAT-deficient macrophages into LDLR-deficient mice showed that ACAT-deficiency in macrophages is sufficient to cause xanthomatosis.
13. Buhman KF, Accad M, Farese RV Jr. Mammalian acyl-CoA:cholesterol acyltransferases. *Biochim Biophys Acta* 2000;1529:142–154. [PubMed: 11111084] The paper discusses the structure of ACAT, the structural and functional relationship and cellular and tissue distribution of *ACAT1* and *ACAT2*, as well as their physiological roles. Two possible models for functions of these enzymes are mentioned. The use of ACAT inhibitors as anti-atherosclerosis drugs is briefly considered and the studies with mouse models of ACAT deficiency are described. The authors ask important questions about ACAT and the use of ACAT inhibitors.

14. Dove ED, Su YR, Swift LL, et al. ACAT1 deficiency increases cholesterol synthesis in mouse peritoneal macrophages. *Atherosclerosis*. 2005 Sept 3; PMID: 16144700 [Epub ahead of print] *ACAT1* deficiency in mouse peritoneal macrophages resulted in increased cholesterol synthesis and efflux, absence of esterification of the newly synthesized cholesterol, dramatic decrease in incorporation of fatty acids into cellular lipids, and higher levels of SREBP1 and LDLR mRNA. Synthesis of phospholipids was not affected. The authors concluded that the up-regulation of cholesterol synthesis occurs at or before the rate-limiting step catalyzed by HMG CoA reductase.
15. Dove DE, Su YR, Zhang W, et al. *ACAT1* deficiency disrupts cholesterol efflux and alters cellular morphology in macrophages. *Atheroscler Thromb Vasc Biol* 2005;25:128–134. The effect of *ACAT1* deficiency in macrophages on cholesterol efflux, cholesterol storage and cellular morphology was studied. *ACAT1*-deficient cells had a lower content of esterified cholesterol, decreased efflux of cellular cholesterol, increased efflux of lipoprotein-derived cholesterol, increased uptake of ³H-cholesteryl ester/acLDL, and dramatically increased *ABCA1* mRNA levels compared with the control macrophages. *ACAT1* deficiency was associated with changes in cellular morphology, such as larger volume of intracellular vesicles.
16. Fazio S, Major AS, Swift LL, et al. RF: increased atherosclerosis in LDL receptor-null mice lacking *ACAT1* in macrophages. *J Clin Invest* 2001;107:163–171. [PubMed: 11160132] In this study, bone marrow transplantation was used to introduce ACAT-deficient macrophages into LDLR-deficient mice. *ACAT*^{-/-}*LDLR*^{-/-} mice developed larger atherosclerotic lesions than *LDLR*^{-/-} mice. The lesions were characterized by higher free cholesterol content and lower macrophage content due to cytotoxic effect of free cholesterol as revealed by TUNEL-staining. The results suggest that total inhibition of ACAT in hyperlipidemia may promote atherosclerosis.
17. Freeman NE, Rusinol AE, Linton M, et al. Acyl-coenzyme A:cholesterol acyltransferase promotes oxidized LDL/oxysterol-induced apoptosis in macrophages. *J Lipid Res* 2005;46:1933–1943. [PubMed: 15995174] The role of ACAT in apoptosis of mouse peritoneal macrophages (MPMs) induced by 7-ketocholesterol (7KC) was investigated, using the ACAT inhibitors and ACAT-deficient macrophages. 58-035, the ACAT inhibitor, prevented the apoptosis of macrophages treated with 7KC as revealed by TUNEL. ACAT-deficient macrophages treated with 7KC and oxLDL showed a 2.4-fold decrease in the percentage of the apoptotic cells compared with the wild-type cells. U18666A, a compound that does not directly affect ACAT but inhibits cholesterol trafficking, suppressed the synthesis of cholesteryl ester without affecting the synthesis of 7KC esters and the ability of 7KC to induce apoptosis in P388D1 cells. When P388D1 cells treated with 7KC were supplemented with either AACOCF₃, an inhibitor of cPLA₂, or ETYA, an inhibitor of arachidonic acid metabolism, the formation of 7KC esters was prevented. Supplementation of P388D1 cells with either oleic acid or arachidonic acid restored the synthesis of 7KC esters, but only arachidonic acid restored the apoptotic effect of 7KC. Based on these results, the authors suggest that one of the roles of ACAT is to induce apoptosis in macrophages in response to OxLDL/oxysterols. 7KC arachidonate may be the molecule inducing apoptosis.
18. Guo Z, Chang CCY, Lu X, et al. The disulfide linkage and the free sulfhydryl accessibility of acyl-coenzyme A:cholesterol acyltransferase 1 as studied by using mPEG₅₀₀₀-maleimide. *Biochemistry* 2005;44:6537–6546. [PubMed: 15850387] In this study, a new method for quantification and mapping of disulfide bonds in human ACAT1, which can also be applied to other proteins, is described. Modification of the cysteines of ACAT1 by mPEG5000-maleimide (PEG-mal), iodoacetamide (IA), and both mPEG-mal and IA under denatured conditions led to the conclusion that of the nine cysteines, two form a disulfide linkage. The use of the mutants in which one cysteine is replaced by alanine suggested that C528 and C546 form a disulfide linkage. Based on the results of the modification of the cysteines of ACAT1 with PEG-mal in microsomal vesicles, the authors concluded that only one cysteine is located in the cytoplasmic part of ACAT1, with the disulfide linkage localized to the lumen of EPR. Enzymatic assay of the Cys to Ala mutants revealed that while none of the cysteins is crucial for enzymatic activity, the disulfide linkage is important in stabilizing the protein *in vivo*.
19. Kellner-Weibel G, Jerome WG, Small DM, et al. Effects of intracellular free cholesterol accumulation on macrophage viability: a model for foam cell death. *Atheroscler Thromb Vasc Biol* 1998;18:423–431. The toxic effects of free cholesterol accumulation in mouse peritoneal macrophages and J774 macrophages were investigated. Inhibition of ACAT was used to load the cell with free cholesterol in the presence and absence of U18666A – a compound known to affect intracellular cholesterol

distribution. Incubation of the cells in the presence of the ACAT inhibitor alone and in the presence of both the ACAT inhibitor and U18666A showed that U18666A neither affects the total free cholesterol content of the cells nor cholesterol efflux, while diminishing the toxic effect of ACAT inhibition. A much smaller number of cells incubated in the presence of U18666A were apoptotic and accumulated fewer cholesterol crystals compared with the cells treated with the ACAT inhibitor alone. The results suggest that the protective effect of U18666A is due to sequestering of free cholesterol in an isolated intracellular pool.

20. Kharbanda RK, Wallace S, Walton B, et al. Systemic acyl-CoA:cholesterol acyltransferase inhibition reduces inflammation and improves vascular function in hypercholesterolemia. *Circulation* 2005;111:804–807. [PubMed: 15699266] Twenty-one human subjects were selected for this study to test the effect of the systemic inhibition of ACAT on hypercholesterolemia. Administration of the systemic ACAT inhibitor, avasimibe, resulted in reduction of circulating levels of TNF- α and cholesterol. Endothelial function of the resistance vessels also improved, as demonstrated by the enhanced responses to acetylcholine, bradykinin and verapamil. This is the first study that showed a positive effect of the systemic ACAT inhibition on inflammation and endothelial function in humans.
21. Kushwaha RS, Vandeberg JF, Rodriguez R, Vandeberg L. Cholesterol absorption and hepatic acyl-coenzyme A:cholesterol acyltransferase activity play major roles in lipemic response to dietary cholesterol and fat in laboratory opossums. *Metabolism* 2004;53:817–822. [PubMed: 15164335] Cholesterol absorption and ACAT activity were measured in high and low-responding opossums. The authors report that high and low-responding opossums kept on a low-cholesterol, low-fat basal diet did not differ in cholesterol absorption. On a high-cholesterol, high-fat diet, however, low-responding opossums showed a decrease in cholesterol absorption. In the case of high-responding opossums kept on a high-cholesterol, high-fat diet, cholesterol absorption increased slightly. Hepatic microsomal ACAT activity increased threefold in high-responding opossums kept on a high-cholesterol, high-fat diet, whereas intestinal microsomal ACAT activity did not change. While strong association was found between plasma cholesterol levels, cholesterol absorption and hepatic ACAT activity, no such association was observed with intestinal ACAT activity.
22. Kusunoki J, Hansoty DK, Aragane K, et al. Acyl-CoA:cholesterol acyltransferase inhibition reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 2000;103:2604–2609. [PubMed: 11382731] The effects of partial inhibition of ACAT by a specific and potent inhibitor F-1394 in apoE-deficient mice maintained on a cholesterol-rich diet were addressed in this study. Partial inhibition of ACAT resulted in reduced macrophage content, smaller atherosclerotic lesion size, and a lower aortic lipid content. No signs of toxicity associated with partial ACAT inhibition were observed.
23. Leon C, Hill JS, Wasan KM. Potential role of acyl-coenzyme A:cholesterol transferase (ACAT) inhibitors as hypolipidemic and antiatherosclerosis drugs. *Pharm Res* 2005;22:1578–1588. [PubMed: 16180116] The focus of this review is ACAT, its role in cholesterol trafficking, and the effect of ACAT inhibitors on atherosclerosis. The authors present the findings that led to the identification of two different ACAT genes, the structure of this transmembrane protein, and cholesterol-binding domains. Expression of ACAT and its regulation as well as the role of ACAT in cholesterol metabolism are discussed. Three broad groups of ACAT inhibitors, their potential use as anti-atherosclerosis drugs, and studies involving ACAT-deficient cells and organisms are described.
24. Matsuo M, Ito F, Konto A, et al. Effect of FR 145237, a novel ACAT inhibitor, on atherogenesis in cholesterol-fed and WHHL rabbits: evidence for a direct effect on the arterial wall. *Biochim Biophys Acta* 1995;1259:254–260. [PubMed: 8541332] The properties of FR 145237 to lower the levels of plasma lipids and reduce atherosclerosis were studied in rabbits maintained on a cholesterol-rich diet and Watanabe heritable hyperlipidemic (WHHL) rabbits lacking LDL receptors. The inhibitor was shown to dose-dependently reduce plasma cholesterol, hepatic total and esterified cholesterol, and the aortic lesion area. The ability of FR 145237 to reduce aortic lesion area was shown to be independent of its plasma cholesterol-lowering properties.
25. Miyazaki A, Sakashita N, Lee O, et al. Expression of ACAT-I protein in human atherosclerotic lesions and cultured human monocytes–macrophages. *Atheroscler Thromb Vasc Biol* 1998;18:1568–1574. The authors of this study showed that ACAT1 of macrophages plays an important during the first stages of the development of atherosclerotic lesions in humans. Highly expressed in human atherosclerotic lesions of aorta as demonstrated by immunohistochemistry, ACAT1 is localized to

- macrophages as shown by double immunostaining. In-vitro studies showed that differentiation of monocytes into macrophages is accompanied by a tenfold increase in ACAT1 protein levels.
26. Murakami S, Ohta Y, Asami Y, et al. The hypolipidemic action of the ACAT inhibitor HL-004 in hamsters fed normal chow. *General Pharmacology* 1996;27:1383–1386. [PubMed: 9304411] The hypolipidemic effect of the ACAT inhibitor HL-004 was examined in male Golden Syrian hamsters. HL-004 was found to be a non-competitive inhibitor of ACAT, preferentially decreasing the activity of the intestine and liver ACAT, with the lowest effect in the adrenal glands. HL-004 dose-dependently decreased total serum cholesterol levels by selectively reducing VLDL cholesterol.
 27. Nicolosi RJ, Wilson TA, Krause BR. The ACAT inhibitor, CI-1011 is effective in the prevention and regression of aortic fatty streak area in hamsters. *Atherosclerosis* 1998;137:77–85. [PubMed: 9568739] The authors of this study report that the ACAT inhibitor, CI-1011, is 50 times more potent than cholestyramine. The effect of CI-1011 on the levels of plasma lipids, liver cholesterol and aortic streak area was studied in six groups of hamsters maintained on a hypercholesterolemic chow-based diet, supplemented with different amounts of the inhibitor. Compared with the control group, the groups receiving CI-1011 showed significant dose-dependent reduction in LDL, triglycerides, total hepatic cholesterol and hepatic cholesteryl esters. The levels of HDL were not affected. Interestingly, the reduction in aortic streak area did not always correlate with the reduction in LDL levels, with the reduction in the former being much more prominent than the reduction in the latter.
 28. Perrey S, Legendre C, Matsuura A, et al. Preferential pharmacological inhibition of macrophage ACAT increases plaque formation in mouse and rabbit models of atherogenesis. *Atherosclerosis* 2001;155:359–370. [PubMed: 11254906] In this study, a series of ACAT inhibitors were screened for their ability to preferentially inhibit macrophage ACAT rather than the liver or intestine ACAT. The following ACAT inhibitors were used: GF1-054, shown to preferentially inhibit the macrophage ACAT; CI-976, a non-specific ACAT inhibitor; GF 1-086, capable of inhibiting both macrophage and liver ACAT; GF 2-021 and GF 2-025, which preferentially inhibited the macrophage ACAT. The inhibitors were tested in three animal models of atherogenesis: fat-fed C57BL/6 mice, chow-fed apoE^{-/-} mice and KHC rabbits. The authors report that macrophage ACAT-specific inhibitors did not reduce foam cell formation but, rather, increased its progression. Reduction in plaque formation, independent of lowering serum cholesterol, was not observed.
 29. Riddell D, Bright CP, Burton BJ, et al. Hypolipidaemic properties of a potent and bioavailable alkylsulphonyl-diphenylimidazole ACAT inhibitor (RP 73163) in animals fed diets low in cholesterol. *Biochem Pharmacol* 1996;52:1177–1186. [PubMed: 8937424] RP 73163 was shown to be a potent, specific and bioavailable inhibitor of ACAT. Its effects on lipid metabolism were studied in rats and rabbits maintained on various diets. In rats fasted overnight, the inhibitor reduced the rate of VLDL secretion, whereas in the fed rats, RP 73163 had no effect on the amount of secreted VLDL but reduced its cholesteryl ester content. In fasted and fed rats, the inhibitor decreased the amount of LDL, without affecting the mass or composition of HDL. *In vitro*, RP 73163 decreased the secretion of apoB by HepG2 cells. In rabbits fed casein, the inhibitor decreased plasma cholesterol levels, reduced the cholesteryl ester content of LDL, while increasing their triglycerides content, and reduced both free and esterified cholesterol levels in the liver.
 30. Rodriguez A, Ashen MD, Chen ES. *ACAT1* deletion in murine macrophages associated with cytotoxicity and decreased expression of collagen type 3A1. *Biochem Biophys Res Commun* 2005;331:61–68. [PubMed: 15845358] This study addressed the effect of *ACAT1* deficiency in mouse peritoneal macrophages. The absence of ACAT1 resulted in increased cytotoxicity, measured by the cellular release of [¹⁴C]adenine, and significantly lower expression of collagen type 3A1. The authors suggest that cytotoxicity associated with *ACAT1* deficiency does not depend on cholesterol enrichment of the cells.
 31. Rodriguez A, Bachorik PS, Wee S. Novel effect of the acyl-coenzyme A:cholesterol acyltransferase inhibitor 58-035 on foam cell development in primary human monocyte-derived macrophages. *Atheroscler Thromb Vasc Biol* 1999;19:2199–2206. The effect of ACAT inhibitors, 58-035 and CI-976, on foam cell development was investigated. Primary human monocyte-derived macrophages were incubated with acLDL in the presence and absence of 58-035 and CI-976 and the cholesterol profile of the cells were examined. The authors report that the inhibitors decreased the total and esterified cholesterol mass in a dose and time-dependent fashion, without causing cytotoxic effects or altering metabolism of the cells. The decrease in total cholesterol mass by 58-035 was due to the

increase in the efflux of free cholesterol and decrease in acLDL uptake. The protein expression of SRs remained unchanged.

32. Rong JX, Kusunoki J, Oelkers P, et al. Acyl-coenzyme A (CoA):cholesterol acyltransferase inhibition in rat and human aortic smooth muscle cells is nontoxic and retards foam cell formation. *Atheroscler Thromb Vasc Biol* 2005;25:122–127. The authors of this study developed a method to transform smooth muscle cells into foam cells using cholesterol–cyclodextrin complexes and studied the effect of ACAT inhibition in the cells by F-1394. Compared with the inhibition of ACAT in macrophages, ACAT inhibition in smooth muscle cells results in little cytotoxicity, indicating that the negative effect of ACAT inhibition *in vivo* may depend on the proportion of macrophages that constitute the atherosclerotic lesion.
33. Sugimoto K, Tsujita M, Wu C, et al. An inhibitor of acylCoA:cholesterol transferase increases expression of ATP-binding cassette transporter A1 and thereby enhances the ApoA-I-mediated release of cholesterol from macrophages. *Biochim Biophys Acta* 2004;1636:69–76. [PubMed: 14984740] In this study, the authors investigated the effect of the ACAT inhibitor, MCC-147, on mRNA and protein levels of ABCA1 and cholesterol efflux from macrophages. MCC-147 did not change *ABCA1* mRNA and protein levels in macrophages not loaded with cholesterol. Treatment of the cholesterol-loaded macrophages with MCC-147, however, resulted in the increase of *ABCA1* mRNA and protein levels in the presence and absence of ApoA-I. Time and dose-dependent increase in cellular-free cholesterol and decrease in esterified cholesterol were observed. MCC-147 caused an increase in the extent of the reversible binding of ApoA-I and ApoA-I-dependent cholesterol efflux.
34. Sugiyama Y, Ishikawa E, Odaka H, et al. TMP-153, a novel ACAT inhibitor, inhibits cholesterol absorption and lowers plasma cholesterol in rats and hamsters. *Atherosclerosis* 1995;113:71–78. [PubMed: 7755657] The effect of TMP-153 on ACAT activity, cholesterol absorption and plasma cholesterol levels in various animals is addressed here. TMP-153 was found to be a potent ACAT inhibitor in hamsters, rats, rabbits and beagle dogs, with IC₅₀ around 5 – 10 nM. The inhibitor significantly decreased cholesterol absorption in the intestine and lowered plasma cholesterol levels in a dose-dependent fashion, without affecting the levels of plasma triglycerides, bile flow and biliary levels of cholesterol.
35. Suguro T, Watanabe T, Kanome T, et al. Serotonin acts as an up-regulator of acylcoenzyme A:cholesterol acyltransferase-1 in human monocyte-macrophages. *Atherosclerosis*. 2005 Sept 9; [Epub ahead of print] In this study, the effect of 5-hydroxytryptamine (5-HT) on *ACAT1* expression and activity in human differentiating monocytes is examined. The authors report a twofold concentration-dependent increase in *ACAT1* expression in differentiating monocytes treated with 5-HT. This increase in *ACAT1* protein levels was accompanied by the increase in *ACAT1* activity. 5-HT also increased *ACAT1* mRNA levels. 5-HT_{2A} receptor was found to be crucial for the effect of 5-HT, as the receptor antagonist, sarpogrelate, completely abolished the effect of 5-HT. The use of a G protein inactivator (GDP-β-S), a protein kinase C inhibitor (roscovitine), a Src family inhibitor (PP2) and mitogen-activated protein kinase inhibitor (PD98059) demonstrated that 5-HT acts via 5-HT_{2A} receptor/G protein/c-Src/PKC/MAPK pathway.
36. Warner GJ, Stoudt G, Bamberger M, et al. Cell toxicity induced by inhibition of acyl coenzyme A:cholesterol acyltransferase and accumulation of unesterified cholesterol. *J Biol Chem* 1995;270:5772–5778. [PubMed: 7890706] Intracellular mechanism of cell toxicity induced by the ACAT inhibitors was investigated in mouse peritoneal macrophages. The cytotoxic effect of the ACAT inhibitors, Sandoz 58-035 and Pfizer CP-113,818, was studied in cholesterol-loaded cells in the absence and presence of either progesterone or U18666A. Both inhibitors induced cytotoxicity, which highly correlated with the accumulation of free cholesterol in the cells. Progesterone or U18666A reversed the cytotoxic effect of the ACAT inhibitors. The authors propose that cytotoxicity is caused by the transport of free cholesterol to the plasma membrane, resulting in its destabilization. Progesterone and U18666A prevent such accumulation by sequestering free cholesterol in intracellular pools.