Roles of acyl-coenzyme A: cholesterol acyltransferase-1 and -2
Ta-Yuan Chang\textsuperscript{a}, Catherine C.Y. Chang\textsuperscript{a}, Song Lin\textsuperscript{a}, Chunjiang Yu\textsuperscript{a}, Bo-Liang Li\textsuperscript{b} and Akira Miyazaki\textsuperscript{c}

Acyl-coenzyme A: cholesterol acyltransferase (ACAT) is an intracellular enzyme that produces cholesteryl esters in various tissues. In mammals, two ACAT genes (ACAT1 and ACAT2) have been identified. Together, these two enzymes are involved in storing cholesteryl esters as lipid droplets, in macrophage foam-cell formation, in absorbing dietary cholesterol, and in supplying cholesteryl esters as part of the core lipid for lipoprotein synthesis and assembly. The key difference in tissue distribution of ACAT1 and ACAT2 between humans, mice and monkeys is that, in adult human liver (including hepatocytes and bile duct cells), the major enzyme is ACAT1, rather than ACAT2. There is compelling evidence implicating a role for ACAT1 in macrophage foam-cell formation, and for ACAT2 in intestinal cholesterol absorption. However, further studies at the biochemical and cell biological levels are needed in order to clarify the functional roles of ACAT1 and ACAT2 in the VLDL or chylomicron synthesis/assembly process. Curr Opin Lipidol 12:289–296. © 2001 Lippincott Williams & Wilkins.

Introduction
In mammals, acyl-coenzyme A: cholesterol acyltransferase (ACAT) is an intracellular enzyme that biosynthesizes cholesteryl esters in a variety of tissues. Two ACAT genes (ACAT1 and ACAT2) have been identified [1–4]. Together, these two enzymes are involved in storing cholesteryl esters as lipid droplets, in absorbing dietary cholesterol, and in providing cholesteryl esters as part of the core lipid for lipoprotein synthesis and assembly. Earlier reviews on this subject have been published [5–11]. The present review focuses on the literature published during the period from January 1999 to January 2001. Suggestions for future research are included at the end of each of the following sections.

Enzymology, mechanism of regulation by cholesterol, and active site analysis
Recombinant human ACAT1 expressed in CHO cells has been purified approximately 7000-fold to homogeneity with retention of catalytic activity [12]. Purified ACAT1 dispersed in taurocholate-based, mixed micelles is fully active. The cholesterol saturation curves of the enzyme assayed in mixed micelles or in phospholipid vesicles are both sigmoidal, supporting the concept that ACAT1 is an allosteric enzyme that is regulated by cholesterol [13]. This work also laid the foundation for study of ACAT at the biochemical and structural biology levels.

The kinetic properties of ACAT2, in terms of its cholesterol and oleoyl coenzyme A saturation curves, as well as its ability to be stimulated by taurocholate micelles, are very similar to those of ACAT1 [14••]. With regard to sterol specificity, when individual sterols were delivered to the enzyme in vesicle form, both ACAT1 and ACAT2 appeared to utilize oxysterols much more efficiently than cholesterol [3]. However, the abilities of the various sterols to transfer from the donor vesicle to the ACAT-containing vesicle vary greatly. These differences could greatly mask the true sterol specificity for the enzyme.

The intrinsic substrate specificity of ACAT remains unknown, but can be studied by dispersing both the enzyme and the substrate in the mixed micelles form [12]. Apart from sterols, the other ACAT substrate is fatty acyl coenzyme A. Miyazaki et al. [15•] demonstrated that hepatic biosynthesis of cholesteryl esters and triglycerides were impaired in mice with a disruption in the gene for stearoyl-coenzyme A desaturase 1, a key enzyme in the biosynthesis of monounsaturated fatty
acid. This finding suggests that, in mouse liver, ACAT favours monounsaturated fatty acid synthesized endogenously as the fatty acyl donor.

Very little information is available at present regarding the ACAT active site. For hamster ACAT1, a serine to leucine mutation (corresponding to serine 269 in human ACAT1) caused the mutant ACAT1 to lose enzyme activity when expressed in CHO cells [16]. This serine is conserved in ACAT2 and in the enzyme diacylglycerol acyltransferase, which is involved in triacylglycerol biosynthesis [17]. Joyce et al. [18**] showed that the same mutation caused loss of ACAT2 activity when assayed in extracts of transfected cells. These findings raise the possibility that this serine residue may constitute part of the active site. This is a tentative interpretation, because the S269L mutation also caused the resultant ACAT1 protein to be expressed at much lower level in transfected cells than the wild-type enzyme [16]. Therefore, more rigorous analysis at the biochemical level is needed before the function of the serine 269 residue can be determined. A superfamily of membrane bound O-acyltransferases, which comprises at least 20 members from different species, has been identified [19*]. The biochemically characterized members of this family encode enzymes that transfer organic acids onto hydroxyl groups of membrane-embedded targets. A histidine (460 in human ACAT1) within a long hydrophobic region is invariant within this family, suggesting that it may be part of the active site. The function of histidine 460 is currently unknown.

In the future, biochemical studies focusing on sterol substrate specificity, mechanisms of sterol dependent allostery, the nature of the active site(s), and the mechanism of catalysis may yield fruitful results.

Membrane topography and oligomeric structure
Hydropathy plots suggest that both ACAT1 and ACAT2 may contain multiple transmembrane segments. In the study reported by Lin et al. [20**], the human ACAT1 protein was individually tagged at various hydrophilic regions with a small, antigenic tag, and then expressed by transfection in mutant CHO cells that lacked endogenous ACAT1. The cells that expressed tagged ACAT1 were rendered permeable with either mild or strong detergent in order to allow selective access of antibodies to the cell interior. Immunofluorescence microscopy was then used to determine the topography of the tagged proteins. The results suggest that human ACAT1 in the endoplasmic reticulum (ER) contain seven transmembrane domains (TMDs).

In a study reported by Joyce et al. [18**], a series of monkey ACAT1 proteins, successively truncated after each predicted TMD, were tagged at the carboxyl-termini with a glycosylation site, and then expressed in microsomal membranes in vitro. The topology of each fusion protein was then determined on the basis of the use of the tagged glycosylation site and accessibility to exogenous protease. The result of this study suggests that ACAT1 contains only five TMDs.

The major difference between these two studies is that the fifth and sixth TMDs (located at the regions comprising residues 325–342 and 471–486) were detectable using the method of Lin et al. [20**], but were not detectable using the method of Joyce et al. [18**]. Both the epitope tag approach and the membrane truncation approach risk altering the topography of the native protein. In the epitope tag approach [20**], all of the tagged proteins were shown to retain full or partial enzyme activities. In contrast, in the truncation approach [18**], whether any of the truncated proteins retained partial enzyme activities was not reported. Using the same truncation approach and the method of immunofluorescence (for identifying the carboxyl-terminus), Joyce et al. [18**] also showed that monkey ACAT2 contains five TMDs. In contrast, using the same epitope tag approach described above, Lin et al. (Lin S, Chang CCY, Chang TY, unpublished data) surprisingly found that human ACAT2 contains only two TMDs. Further studies are needed to clarify the discrepancy regarding the topographic arrangements of both ACAT1 and ACAT2.

Both ACAT1 and ACAT2 are integral membrane proteins. Determining the oligomeric state of an integral membrane protein requires multifaceted approaches. By using coimmunoprecipitation, radioimmunoprecipitation, chemical cross-linking, and gel filtration chromatography and sucrose density gradient centrifugation in H2O and D2O, Yu et al. [21**] showed that human ACAT1 mainly exists as a homotetramer in intact cells and in vitro. The oligomeric state of ACAT2 is currently unknown. The relationship between the oligomeric state of ACAT and its catalytic efficiency remains to be investigated.

Tissue distribution, and changes in intracellular localization regulated by various lipoproteins
Antibodies that are specific to either ACAT1 or ACAT2 have been used to perform tissue distribution studies. In adult human tissues, ACAT1 protein has been found in almost all of the cell types and tissues examined, including liver hepatocytes and Kupffer cells, adrenal, skin cells, intestinal enterocytes, neurons, and macrophages [14**,22,23**]. In contrast, ACAT2 protein is mainly found in the apical region of the intestinal villi; it is also present in hepatocytes, but at low levels. Surprisingly, in fetal human livers, relatively high levels
of ACAT2 were found [14**]. In mice and monkeys, the relative tissue distributions of ACAT1 and ACAT2 are similar, but not identical to those in humans [24,25**,26**]. The major difference is that, in mouse and monkey hepatocytes ACAT2 is the major isoenzyme, whereas in adult human hepatocytes ACAT1 is the major isoenzyme. Species differences in tissue expression levels of other enzymes/proteins that are involved in lipoprotein metabolism have previously been reported.

It is known that the ACAT-specific activities in human liver microsomes are much lower than those found in liver microsomes prepared from several other mammalian species, including rabbit, rat, mouse, and pig [14**,27,28] (Chang T-Y et al., unpublished data). In parallel, functional LDL receptor (LDLR) activity in human liver is believed to be much lower than that in mouse liver. The latter finding was derived from the fact that the plasma LDL turnover is much faster in mice than it is in humans; the half-life of LDL in mice is approximately 1.5 h [29], whereas in normal humans it is approximately 1.5 days [30].

On the basis of these results, we formulated the following hypothesis. The LDL-dependent intracellular cholesterol trafficking activities may be much lower in human liver than in mouse liver. In adult human liver ACAT1 may be constitutively expressed, and its expression is sufficient to cope with the normally low level of cholesterol trafficking and turnover, etc. In contrast, the expression of hepatic ACAT2 may be inducible by various pathophysiologic/physiologic stimuli that trigger higher levels of cholesterol trafficking and turnover. The latter situation may occur frequently in human fetal liver. Figure 1 summarizes our current understanding of ACAT1/ACAT2 distribution in adult human tissues.

In a variety of cell types, ACAT1 is found to be mainly located in the ER [23**,32]. In macrophages, electron microscopy studies [23**] showed that it is mainly associated with membranes of the rough ER; in addition, a small portion of ACAT1 is located in vesicles that are believed to be derived from the ER [33*]. In mouse macrophages maintained in suspension, a small portion of ACAT1 is associated with the plasma membrane fraction [34]. It has been shown that treatment of macrophages with acetylated LDL or oxidized LDL (or other atherogenic lipoprotein) causes significant increase in cholesteryl ester synthesis, whereas treatment of macrophages with HDL (an antiatherogenic lipoprotein) inhibits cholesteryl ester synthesis. In human macrophages, Sakashita et al. [23**] showed that acetylated LDL did not alter the total ACAT1 protein content in the cells; instead, it caused ER to vesiculate and produced vesicles of 80–150 nm in diameter. A significant portion (up to 30%) of ACAT1 was found to be located in these small vesicles. In a related study, Li and Pownall [35**] showed that loading mouse macrophages with HDL caused a significant portion of ACAT1 to appear in vesicles and in the cell periphery adjacent to the plasma membrane. Together, these findings strongly implicate that, in macrophages, a novel ER vesiculation process, or an ACAT1 translocation process, may be involved in mediating the regulation of ACAT1 activity by various lipoproteins.

ACAT2 is mainly expressed in intestinal enterocytes and in hepatocytes. Its exact location remains to be determined. In transfection studies, when expressed in CHO cells ACAT2 was located mainly in the ER [18**]. Chang et al. (Chang CCY, Lin S, Chang TY, unpublished data) found that, in differentiated Caco-2 cells (a cell type that resembles the intestinal enterocytes), ACAT1 and ACAT2 are localized in distinct subcellular compartments. The mode(s) of regulation of ACAT2 by various agents remains largely unknown.

It is possible that, within a polarized cell, ACAT1 and ACAT2 are located at different intracellular membranes in order to serve the purpose of esterifying cholesterol from various sources. The molecular events that are involved in converting intracellular cholesteryl esters as part of the core lipid in VLDL or in chylomicrons remain largely unknown. Therefore, at present it is difficult to identify the precise role(s) of ACAT1 and ACAT2 in the process of lipoprotein synthesis and assembly. In the future, single-cell types that express both ACAT1 and ACAT2, including Caco-2 and the HepG2 cells, may serve as appropriate models in which to define further the biologic functions of ACAT1 and ACAT2 at the cellular level.

**Low-density lipoprotein receptor, fatty acids, and ACAT**

It has been hypothesized [36] that unsaturated fatty acids, by enhancing ACAT activity, may reduce the amount of free cholesterol pool that regulates the LDLR expression at the transcription level. To test this hypothesis, Spady et al. [31**] transiently over-expressed human ACAT1 in hamster livers (by adenovirus-mediated gene transfer). This procedure caused an increase in cholesterol esterification, which led to a compensatory increase in de-novo cholesterol synthesis, but no induction of LDLR expression was observed, suggesting that fatty acids regulate LDLR expression via mechanisms that are independent of ACAT.

**Gene structure and regulation at the gene expression level**

A 10-base-pair sequence termed the sterol-regulatory element has been identified within the promoter regions
of a variety of sterol-responsive genes, including the LDLR and 3-hydroxy-3-methylglutaryl coenzyme A reductase genes, among others [37]. In humans, the sterol-regulatory element could not be identified within either the ACAT1 promoter [38] or the ACAT2 promoter [39]. This finding is consistent with the concept that the main mode of sterol-dependent regulation of ACAT is by allosteric control, and not by transcriptional control [6].

Unlike almost all other known human genes, the human ACAT1 gene is located in two different chromosomes (1 and 7), with each chromosome containing a different ACAT1 promoter (P1 and P7) [38]. In all human tissues and cell lines examined thus far, Northern analyses have revealed the presence of four ACAT1 messenger RNAs (7.0, 4.3, 3.6, and 2.8 kilonucleotides). These messages share the same short 5’-untranslated region and coding sequence. The 4.3-knt message contains an additional 5’-untranslated region (exons Xa and Xb; 1289 nt in length) upstream from the exon 1 sequence. Analyses showed that the 2.8-knt and 3.6-knt messages are produced from the P1 promoter, whereas the 4.3-knt messenger RNA is produced from two different chromosomes by a novel RNA recombination event that presumably involves trans-splicing. The functional significance of this finding is still unknown, but is under investigation at present. The P1 promoter is contiguous with the coding sequence, whereas the P-7 promoter is contiguous with the long and optional exon Xa (1277 base pairs).

ACAT1 is highly expressed in human atherosclerotic lesions, particularly in macrophage-derived foam cells...
ACAT inhibitors as potential drugs, and gene alteration studies in mice

During the past 20 years, various classes of compounds that act as ACAT inhibitors have been developed (for review [45–47]). Recently, a few of these candidate drugs have reached the stage of clinical trials as potential lipid-lowering and antiatherosclerotic agents. A large volume of literature on ACAT inhibitors exists. Due to space limitations, only a few selected studies are reviewed here.

Essentially all of the ACAT inhibitors that have been examined thus far inhibited both ACAT1 and ACAT2 in vitro with similar potency (for example [14**]). In various animal models, ACAT inhibitors have been shown to cause decreases in chylomicron, VLDL, and LDL levels. In addition, in certain animal models of atherosclerosis, ACAT inhibitors caused decreases in macrophage contents and led to the reduction in atherosclerotic lesions [46,47].

A potentially new clinical application of ACAT inhibitors has been described by Aragane et al. [48**], who used the balloon injury model in rabbits to study the effect of hypercholesterolemia on intimal hyperplasia. Their results suggest that the presence of cholesterol ester-loaded macrophages preceded the intimal thickening and smooth muscle cell proliferation. Furthermore, treating these rabbits with the specific ACAT inhibitor F-1394 caused significant reduction in intimal thickening in the lesion, raising the possibility that ACAT inhibitor may be beneficial for treating restenosis after angioplasty in hyperlipidemic patients. In a separate study, using a rat model, Post et al. [49] showed that the ACAT inhibitor avasimibe (CI-1011) stimulated bile acid synthesis and cholesterol 7a-hydroxylase in cultured rat hepatocytes and in vivo. These observations may explain in part the potent cholesterol-lowering effects of avasimibe in the rat.

Sulfonylureas, including glibenclamide, have been used clinically to treat noninsulin-dependent diabetes mellitus. At nanomolar concentrations, glibenclamide stimulates insulin secretion in pancreatic β-cells. Ohgami et al. [50] showed that at much higher concentrations (with a 50% inhibitory concentration value of 20 μmol/L), glibenclamide is also an ACAT inhibitor, raising the possibility that sulfonylurea may provide a prototype for a new generation of ACAT inhibitors.

When HDL or another cholesterol acceptor was present in growth medium, ACAT inhibitors promoted cellular cholesterol efflux [51–53]. On the other hand, other investigators showed that, if no cholesterol acceptor was present in growth medium, ACAT inhibitors caused cholesterol-loaded mouse peritoneal macrophages to undergo apoptosis and necrosis [54–56]. These studies suggest that it may be safer and more beneficial to use ACAT inhibitors for treating patients with atherosclerosis in conjunction with agent(s) that raise blood HDL levels.

ACAT1-null (ACAT1−/−) mice have been produced by targeted gene disruption [57,58**]. In these mice, ACAT activities were completely absent in adrenal, testes, ovaries, and macrophages, but were amply present in livers and intestines. Lack of ACAT1 does not appear to cause major pathophysiologic symptoms in these mice. In order to test the effect of ACAT1 deficiency in atherosclerosis, the ACAT1−/− mice were employed to produce double knockout mice that lacked both ACAT1 and apolipoprotein E, or that lacked both ACAT1 and LDLR. Unfortunately, the outcomes of those studies were not able to demonstrate clearly whether ACAT1 deficiency attenuates atherosclerosis in mice [58**,59**]. Instead, high fat feeding of these double knockout mice led to unexpected findings; they suffered from extensive cutaneous xanthomatosis, cholesterol accumulation in the skin, skin loss, and dry eye. In order to investigate these observations further, marrow from ACAT1−/−/LDLR−/− mice was transplanted into LDLR−/− recipients; this procedure led to xanthoma in the recipient mice, indicating that ACAT1 deficiency in macrophages played a key role in the skin pathology observed in these mice. Those studies indicate that ACAT1 inhibitors may be associated with severe side
effects. In the future, in safety studies that involve the use of ACAT inhibitors in animals and in humans, the symptoms that were observed in the double knockout mice should be carefully monitored for.

It is worth pointing out that the results of the ACAT1 gene inactivation studies in mice predict the outcomes of an ACAT1 inhibitor used at extremely high dosages. Experimentally, when tested in beagle dogs at moderate dosages, the ACAT inhibitor avasimibe (CI 1011) appeared well tolerated, and without many side effects [60]. When tested in rats, rabbits, or guinea pigs [61], another ACAT inhibitor F 12511 was also well tolerated. In addition, because the ACAT1/ACAT2 distribution in humans is different from that in mice, it is possible that the symptoms of ACAT1 deficiency observed in the double knockout mice may be species-specific events.

The fact that certain tissues still express ample ACAT activities in the ACAT1−/− mice, coupled with the finding that two ACAT-like genes were found in yeast [62,63], led to the discovery of the second mammalian ACAT gene (ACAT2) [2–4]. Recently, ACAT2−/− mice have been produced [25]. These mice lack ACAT activity in intestine and liver. ACAT2 deficiency has profound effects on cholesterol metabolism in mice fed a cholesterol-rich diet, including resistance to hypercholesterolemia and cholesterol gallstone formation. This study provides the genetic proof that ACAT2 plays an important role in dietary cholesterol absorption in mice, and an ACAT2 inhibitor at high dosage will be effective in reducing the amount of cholesterol absorbed under high-fat/high-cholesterol diets.

This conclusion probably can be safely extended to other mammalian species including humans, because in humans and in monkeys ACAT2 has been shown to be localized at the apical region of the small intestines [14,26]. The ACAT gene knockout study also raises the exciting possibility that ACAT inhibitors may be protective against cholesterol stone formation in humans. In humans, ACAT1 is the major isoenzyme in liver, including hepatocytes and the hepatic duct cells [14], and ACAT2 is the major isozyme in intestine. Thus, in humans, using a general ACAT inhibitor that acts on both ACAT1 and ACAT2 for gallstone treatment may be more beneficial than using a specific ACAT2 inhibitor. Inhibiting ACAT1 activity in liver may result in availability of more free cholesterol as a substrate for bile acid synthesis, thus increasing the capacity of bile salt for cholesterol dissolution in the bile; inhibiting ACAT2 activity in the intestines may reduce the amount of dietary cholesterol absorbed that contributes to the biliary cholesterol pool.

Conclusion
The reaction catalyzed by ACAT represents a prototype for a group of membrane-bound enzymes that transfer the fatty acyl moiety from fatty acyl-coenzyme A to the hydroxyl group of a lipophilic substance embedded in the lipid bilayer. It is thus important to continue the structure–function analysis of ACAT at the biochemical and structural biology levels. ACAT1 and ACAT2 have similar but different biochemical characteristics, rendering them tissue-specific targets for drug development. Gene inactivation studies have produced convincing evidence for tissue-specific functions of ACAT1 and ACAT2 in mice. Other findings indicate that the ACAT1/ACAT2 distribution in the liver is species dependent. In normal adult humans, ACAT1 is the major isoenzyme in hepatocytes and macrophages, whereas ACAT2 is the major isoenzyme in the apical region of small intestines. We propose that in macrophages, ACAT1 plays a critical role in foam-cell formation; in intestinal enterocytes, ACAT2 plays a critical role in the cholesterol absorption process; ACAT1 and ACAT2 work in concert to participate in the chylomycin synthesis/assembly process. In hepatocytes, the total pool of cholesteryl esters produced by both ACAT1 and ACAT2 serve as a determinant in the VLDL synthesis/assembly process. To test this hypothesis, it will be important to define the biologic functions of ACAT1 and ACAT2 in single cell types that express both isoenzymes.

Acknowledgements
Research carried out in the authors’ laboratories was supported by Grants from National Institutes of Health, USA (HL 36709 and HL 60306 to T-Y.C.), National Natural Scientific Foundation of China (39425005 to B-L.L.), and a Grant-in-Aid for Scientific Research (12671118) from the Ministry of Education, Science, Sports and Culture of Japan. T-Y.C. thanks Dr Michael S. Brown for helpful discussions.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

Roles of ACAT1 and ACAT2: Chang et al.


This paper showed that, in humans, the ACAT1 gene is located in two different chromosomes. This is an unprecedented finding in human gene analyses. One of the messenger RNAs is believed to be produced through a novel RNA recombination mechanism that involves trans-splicing.
This paper provides a rationale to explain the significant cholesterol-lowering effect observed in a rat model. Bernard DW, Rodriguez A, Rothblat GH, Glick JM. Influence of high density lipoprotein on esterification of cholesterol in macrophages and hepatoma cells. Arteriosclerosis 1990; 10:135–144.

This paper, along with those by Meiner et al. [57] and Accad et al. [59], provided the genetic proof for the functional importance of ACAT1 in mouse macrophages. It also raised the caution that ACAT1 inhibitor used at extremely high dosage resulted in various undesirable cytotoxicities. Robertson DG, Breider MA, Milad MA. Preclinical safety evaluation of avasimibe in beagle dogs: an ACAT inhibitor with minimal adrenal effects. Toxicol Sci 2001; 59:324–334.

This paper, along with that by Junquero et al. [61], demonstrated that ACAT inhibitors used at moderate dosages produced minimal toxicities in the various animal models tested. Robertson D, Oms P, Cailla-Durand E, et al. Pharmacological profile of F 12511, an ACAT inhibitor. Arterioscler Thromb Vasc Biol 1999; 19:2199–2206.

This paper, along with that by Junquero et al. [61], provided the genetic proof for the functional importance of ACAT1 in mouse macrophages. Hakamata H, Miyazaki A, Sakai M, et al. Species difference in cholesteryl ester cycle and HDL-induced cholesterol efflux from macrophage foam cells. Arterioscler Thromb 1994; 14:1860–1865.

This paper, along with that by Junquero et al. [61], provided the genetic proof for the functional importance of ACAT1 in mouse macrophages. Hakamata H, Miyazaki A, Sakai M, et al. Species difference in cholesteryl ester cycle and HDL-induced cholesterol efflux from macrophage foam cells. Arterioscler Thromb 1994; 14:1860–1865.

This paper, along with those by Meiner et al. [57] and Accad et al. [59], provided the genetic proof for the functional importance of ACAT1 in mouse macrophages. It also raised the caution that ACAT1 inhibitor used at extremely high dosage resulted in various undesirable cytotoxicities. Robertson DG, Breider MA, Milad MA. Preclinical safety evaluation of avasimibe in beagle dogs: an ACAT inhibitor with minimal adrenal effects. Toxicol Sci 2001; 59:324–334.

This paper, along with that by Junquero et al. [61], demonstrated that ACAT inhibitors used at moderate dosages produced minimal toxicities in the various animal models tested. Robertson D, Oms P, Cailla-Durand E, et al. Pharmacological profile of F 12511, an ACAT inhibitor. Arterioscler Thromb Vasc Biol 1999; 19:2199–2206.