thematic review

Carboxyl ester lipase: structure-function relationship and physiological role in lipoprotein metabolism and atherosclerosis

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Abstract **Carboxyl ester lipase (CEL), previously named cholesterol esterase or bile salt-stimulated (or dependent) lipase, is a lipolytic enzyme capable of hydrolyzing cholesteryl esters, tri-, di-, and mono-acylglycerols, phospholipids, lysophospholipids, and ceramide. The active site catalytic triad of serine-histidine-aspartate is centrally located within the enzyme structure and is partially covered by a surface loop. The carboxyl terminus of the protein regulates enzymatic activity by forming hydrogen bonds with the surface loop to partially shield the active site. Bile salt binding to the loop domain frees the active site for accessibility by water-insoluble substrates. CEL is synthesized primarily in the pancreas and lactating mammary gland, but the enzyme is also expressed in liver, macrophages, and in the vessel wall. In the gastrointestinal tract, CEL serves as a compensatory protein to other lipolytic enzymes for complete digestion and absorption of lipid nutrients. Importantly, CEL also participates in chylomicron assembly and secretion, in a mechanism mediated through its ceramide hydrolytic activity. Cell culture studies suggest a role for CEL in lipoprotein metabolism and oxidized LDL-induced atherosclerosis. Thus, this enzyme, which has a wide substrate reactivity and diffuse anatomic distribution, may have multiple functions in lipid and lipoprotein metabolism, and atherosclerosis.—** Hui, D. Y., and P. N. Howles. **Carboxyl ester lipase: structure-function relationship and physiological role in lipoprotein metabolism and atherosclerosis. *J. Lipid Res.* 2002.** 43: **2017–2030.**

Supplementary key words lipid absorption • cholesterol absorption • atherosclerosis • chylomicron assembly • reverse cholesterol transport

Carboxyl ester lipase (CEL), previously named pancreatic cholesterol esterase and also known as bile salt-stimulated (or -dependent) lipase, is a nonspecific lipolytic enzyme capable of hydrolyzing cholesteryl esters, tri-, di-, and mono-acylglycerols, phospholipids, lysophospholipids, and ceramide (1–3). The hydrolysis of water-insoluble carboxyl esters with long chain fatty acyl groups by CEL requires its activation by bile salt. However, CEL hydrolysis of water-soluble substrates such as carboxyl esters with short chain fatty acids or lysophospholipids does not have an absolute dependence on bile salt activation. The CEL protein is synthesized primarily in the pancreatic acinar cells and lactating mammary glands of higher mammals. Small amounts of CEL are found in other tissues, particularly the liver, macrophages, endothelial cells, and eosinophils (4–10). Extensive research has been performed in recent years on the structure-function relationship of this protein as well as its physiological role in lipid metabolism. A significant body of work has also been devoted to studying CEL processing and transport in pancreatic acinar cells. The latter topic was reviewed recently (11) and will be discussed only briefly in this article when appropriate. This article will present an updated review on the structure-function studies of CEL as well as an in-depth discussion on the current understanding of its role in lipid metabolism.

PROTEIN STRUCTURE-FUNCTION RELATIONSHIP

Active site

The nucleotide sequence of CEL from various species shows that it is a highly conserved protein belonging to the α/β hydrolase family. The carboxyl ester hydrolytic activity is conferred through a catalytic triad with Ser-His-Asp, forming a charge relay network for substrate hydrolysis. The serine residue at position 194 is responsible for initiating nucleophilic attack on the substrate carboxyl ester bond (12), with histidine-435 participating in an acid-base catalysis reaction on the substrate carbonyl to form an acyl-enzyme intermediate (13–15). The aspartic acid

Abbreviations: CEL, carboxyl ester lipase; lysoPC, lysophosphatidylcholine; PTL, pancreatic triglyceride lipase.

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at position 320 constitutes the third component of the catalytic triad and provides a better acid-base reaction through modulation of the reaction pK_a (15, 16). The CEL protein is also capable of hydrolyzing lipoamides (17) and releasing fatty acid from ceramide (18, 19). Interestingly, the lipoamidase activity of CEL is dependent on a functional serine-194, but not histidine-435 (17). Thus, the precise mechanism by which CEL hydrolyzes ceramide and other lipid amides remains to be determined.

X-ray crystal structure

Recent X-ray crystallography data have provided additional insights into the protein structure of CEL. A truncated bovine CEL lacking the proline-rich repeating units at the carboxy-terminus was shown at 0.28 nm resolution to be a protein with 13 β-strands and 14 α-helices (20). However, a full-length bovine CEL structure resolved at 0.16 nm resolution indicated a protein with 11 β-strands and 15 α-helices (21). The X-ray crystal structure of a truncated human CEL showed that this protein is built around a core comprised of strongly twisted 11-stranded β-sheets, with a smaller three-stranded β-sheet found at the amino-terminus (22). All crystal structure data showed that the active site triad of CEL is centrally located within the enzyme, similar to that observed in other lipases and esterases. The difference between CEL and other serine esterases is restricted to the polypeptide segments connecting the conserved β-strands of the structural scaffold (22). The CEL protein has an apparent insertion at positions 115–126, acts as a surface loop that sits on top of the opening to the catalytic triad. This active site loop is distinctly different from the lid structure that covers the active sites of other lipases. The lid structure of these latter enzymes protects the hydrophobic surface surrounding the active site from the aqueous environment, and its displacement is required for maximal enzyme activity at the water-lipid interface (23). In contrast, the surface loop of the truncated CEL is mobile and assumes an open conformation even in the absence of a lipid phase (22). Accordingly, the active site of truncated CEL is in a predominantly open and solvent-exposed conformation, allowing direct access to water-soluble substrates. However, full-length glycosylated forms of CEL may have the loop in a closed conformation that restricts substrate access through this pocket. Another hydrophobic patch that is exposed to solvent includes tryptophan-103, tyrosine-105, leucine-124, tryptophan-227, leucine-282, valine-285, isoleucine-323, phenylalanine-324, leucine-392, phenylalanine-393, isoleucine-439, and phenylalanine-443 (22). This region may allow access to the active site by water-soluble substrates.

Bile salt binding

The hydrolysis of water-insoluble substrates by CEL requires bile salt containing 3α,7α-hydroxy groups (e.g., cholate or chenodeoxycholate and their conjugates) (24). Chemical modification studies showed that arginine residues in CEL are important for its interaction with bile salt (25, 26). However, the x-ray crystal structure of bovine CEL-taurocholate complex demonstrated CEL-bile salt interaction but failed to identify the arginine residues involved (20). Interestingly, X-ray crystallography of truncated human CEL in the presence of the bile salt analog CHAPS failed to detect CEL-bile salt interaction (22). The latter result suggests that deletion of the proline-rich repeats may compromise the ability of CEL to interact with bile salt.

In an effort to identify the bile salt interacting site in CEL, our laboratory utilized a mutagenesis approach to explore whether arginine-63 and arginine-423 participate in this process. These amino acid residues were highlighted previously as possible bile salt binding sites by computer modeling analysis of the CEL structure (27). Our results showed that mutagenesis of arginine-63 and arginine-423 to alanine and glycine, respectively, significantly reduced the bile salt stimulated cholesteryl ester hydrolytic activity of CEL (28). Importantly, these mutations did not affect the bile salt-independent lysophospholipid hydrolytic activity of CEL (28). Therefore, although arginine-63 and arginine-423 are not directly involved with the catalytic site of CEL, these residues are important for modulating bile salt activation of CEL. Moreover, the R63A and R423G mutant forms of CEL were similar to native CEL in monomeric bile salt-induced changes in circular dichroism spectra (28, 29). Thus, arginine-63 and arginine-423 residues are not involved with CEL interaction with monomeric bile salt. It is likely that the decreased hydrolytic activity of the arginine-63 and arginine-423 mutants is due to alterations in the positive-charged domains necessary for CEL interaction with negatively charged micelles carrying the cholesteryl ester substrate. This hypothesis is consistent with the crystal structure data of the bovine CEL (20), which shows that arginine-63 and arginine-423 are key residues forming the two parallel rows of positively-charged residues near the active site domain of CEL.

One interesting observation made during characterization of the arginine-63 and arginine-423 mutants is that they have increased hydrolytic activity against water-soluble substrates over the native enzyme (28). As discussed above, the surface loop in full length CEL may serve as a partial lid in limiting substrate accessibility to the active site. Thus, one potential explanation for the increased lipolytic activity of the arginine mutants is that the mutations resulted in subtle changes in protein conformation, favoring increased accessibility of the active site to water-soluble substrates. This hypothesis is supported by the crystal structure of the bovine CEL; in the absence of bile salt, the carboxy-terminus is lodged in the active site of the enzyme (21). Computer modeling of the domain structure surrounding the loop and the partial lid domains of CEL showed that arginine-63 is hydrogen-bonded to serine-121 (lysine in the rat sequence), which holds the loop/lid in place and forces the neighboring methionine-111 to form a hydrogen bond with the carboxy-terminus, lodging it within the active site (28). The binding of micellar bile salt to the loop domain of CEL would disrupt the

hydrogen bonding between arginine-63 and serine-121, causing the loop to peel back and the carboxy-terminus to be freed from the active site. This allows for substrate access to the hydrophobic pocket. Mutation in arginine-63 abolishes micellar bile salt binding, resulting in an enzyme that is not activated by bile salt. However, arginine-63 mutation also disrupts hydrogen bonding between residues 63 and 121, partially freeing the carboxy terminus from the active site domain and producing an enzyme with increased bile salt-independent activity. Arginine-423, which is located in close proximity to the active site groove, may serve a similar purpose. Its mutation to a neutral amino acid residue is also expected to abolish hydrogen bonding patterns resulting in peeling back of the loop domain and freeing the C-terminus from the active site domain (28). A ribbon diagram depicting the CEL structure in the absence or presence of bile salt is shown in **Fig. 1**. The crystal structure of the human enzyme, which identifies a residue in close proximity to arginine-423 (lysine-429), near the edge of a large concave surface opposite the active site domain (22) is consistent with this model.

Proline-rich repeats

The recent data demonstrating the participation of the carboxyl terminal domain of CEL in modulating its enzymatic activity is potentially of importance considering that this is the least conserved domain among CEL from various species (3). The human enzyme is the largest in the group, and is highly polymorphic with molecular weights ranging from 120- to 140-kDa. The rat, mouse, bovine, and rabbit CEL are considerably smaller with an approximate molecular weight of 74 kDa. The difference in size among the various CEL can be attributed to the different number of proline-rich repeating units at the carboxyl terminus of the protein. The prototype human CEL contains 16 repeating units with the consensus sequence of PVPPPTDDSQ (30). The rat, mouse, bovine, and rabbit enzymes contain four, three, and two such repeating sequences. Importantly, variant forms of human CEL with 3, 6, 16, and 28 proline-rich repeating units have been reported (31, 32). The variant CELs are not rare mutations but the polymorphisms are widely distributed among the population (31, 32). In one study of 295 healthy subjects, only 44% of the subjects were found to be homozygous for the CEL gene with 16 proline rich repeats, and 56% of the individuals carry at least one polymorphic form of the CEL (32). Whether the number of proline-rich repeating units can dictate CEL activity, particularly against water-soluble substrates, has not been determined. Interestingly, these proline-rich repeats are the site of *O*-glycosylation and are important for maintaining protein stability (33). Thus, the number of proline-rich repeats in CEL may also be important for intracellular processing of the enzyme prior to its secretion, as well as in determining its stability after its secretion into an aqueous environment. Accordingly, polymorphisms in the number of proline-rich repeating units may also dictate the amount of protein being produced. Considering the increasing evidence for the presence of CEL in anatomic sites with minimal bile salt (see below for discussion), the physiological significance of the CEL polymorphism needs to be addressed.

CARBOXYL ESTER LIPASE GENE STRUCTURE AND EXPRESSION

Gene structure

Structural organization of the human, rat, and mouse carboxyl ester lipase gene is well conserved with 11 exons interrupted by 10 introns (3). Each exon delineates a key structural and/or functional domain of the carboxyl ester lipase protein. For example, exon 1 encodes the signal peptide sequence of the protein. Exon 3 contains the first intramolecular cysteine crosslink, whereas the second intramolecular cysteine crosslink is encoded in exon 7 of the carboxyl ester lipase gene. The active site triad of serine-194, histidine-435, and aspartic acid-320 are encoded within exons 5, 8, and 10, respectively. The surface loop that partially covers the active site groove is encoded by sequence within exon 4. The least conserved exon among the carboxyl ester lipase genes of various species is exon 11, which encodes the *O*-glycosylation domain and the proline-rich repeating units at the carboxy-terminus of the protein. The major difference among nucleotide sequences in exon 11 is the number of proline-rich repeating units. As discussed previously, the rat and mouse carboxyl ester lipase genes encode proteins with four and three proline-rich repeating units, respectively. In contrast, the human gene is highly polymorphic, with the most common allele encoding a protein with 16 proline-rich repeats. The high frequency of polymorphism is due to the presence of a hypervariable region in the 3′-end of the gene (34). The 5′-flanking sequence of the CEL gene contains the classical TATA and CCAAT elements implicated in tissue-specific expression of the gene (35). The upstream sequence also contains consensus sequence for AP1, AP2, and SP1 binding sites. In addition, putative sterol, glucocorticoid, and acute phase response elements can also be identified in the regulatory region of the CEL gene (35). However, whether these *cis* acting elements function in regulation of CEL gene expression has yet to be determined. Thus, additional studies aimed at determining the functional significance of these regulatory domains are warranted. Results from these studies will shed additional light into the role of CEL in physiology and pathophysiology.

Gene expression

The major tissue for CEL biosynthesis is the pancreas. Its expression is low in immature animals and increases during the maturation of the pancreas (36). The CEL gene is also expressed at high level in lactating mammary glands (37–40). The mammary-derived CEL is found in abundance in milk and is postulated to substitute for the pancreatic enzyme in aiding nutrient digestion and absorption in newborns prior to the maturation of the pancreas (see below). The expression of the CEL gene in exo-

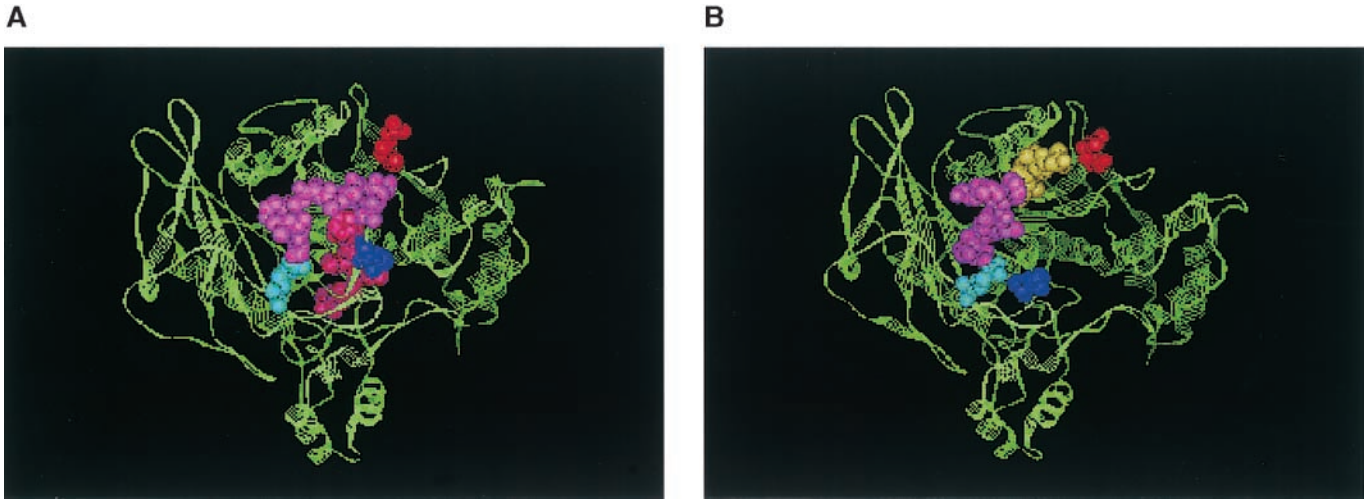

Fig. 1. Ribbon diagram of the carboxyl ester lipase (CEL) model in the absence (A) or presence (B) of taurocholate. The magenta color denotes the hairpin loop; cyan highlights the Arg^{63} residue, orange highlights the Arg^{423} residue; Met^{111} is highlighted in blue; red color outlines the C-terminal residues; and yellow color depicts a taurocholate molecule. (Reproduced from Reference 28, and used by permission of the American Society for Biochemistry and Molecular Biology.)

crine pancreas of rodents is dependent on pancreatic transcription factor 1 (PTF-1) interaction with specific PTF-1 binding site in the 5′-promoter region of the CEL gene (40, 41). The effect of PTF-1 binding to this site on rodent CEL gene expression is further augmented by PTF-1 binding to a more distal PTF-1 binding site and by a factor that binds to the mouse distal promoter element mDPE (41). The human CEL gene also contains the essential PTF-1 binding element located at a position corresponding to the essential PTF-1 site in the mouse promoter. However, PTF-1 does not interact with this promoter sequence in the human CEL gene (41). The difference is due to the presence of an overlapping CCAAT/enhancer-binding protein (C/EBP)-like binding motif in the human gene sequence that interferes with PTF-1 binding to the same site (41). High-level expression of the human CEL gene in the pancreas is achieved by another pancreas-specific enhancer element found in the 5′-upstream sequence of the CEL gene (42).

Regulatory elements responsible for CEL expression in other tissues are less well characterized. As discussed previously, CEL is highly expressed in lactating mammary glands of most species, except cows and rats. A tissue-specific mammary gland factor recognition sequence has been identified in the 5′-upstream region of the human gene (43). In addition, an 11-bp sequence that is present in the promoter region of other milk protein genes is also present in the regulatory region of the human CEL gene (43–45). Whether these consensus sequences play a role in CEL expression in lactating mammary glands remains to be determined.

The CEL gene is also expressed at low levels in liver and in human macrophages. Experiments with reporter gene constructs transfected into HepG2 cells showed that 5′-flanking sequence spanning nucleotides –10 to –930 is required for hepatic CEL expression (35). Human macrophage expression of the CEL gene requires the interaction of upstream stimulatory factors (USF) 1 and 2 with an E-box domain located at −47 to −52 of the human CEL gene (46). However, while the E-box motif is necessary for transcriptional activity in human monocyte/macrophages, this motif cannot independently induce CEL gene transcription and an additional nuclear receptor binding site at −60 to −66 is required (46). Interestingly, the E-box sequence is apparently also important for CEL expression in pancreas and in mammary glands (46), although additional tissue specific elements are also required for CEL expression in these tissues. The macrophage-specific transcription factor that works with E-box binding proteins in promoting CEL expression in this cell type has yet to be identified.

DIETARY LIPID ABSORPTION

The role of CEL in dietary fat and cholesterol absorption has been an enigma for nearly four decades. While cell culture and other in vitro studies have thoroughly defined the potential functions of CEL in the digestion and intestinal absorption of cholesteryl ester, phospholipid, and triglyceride (47–49), only its cholesteryl ester hydrolytic activity is unique to this enzyme in the digestive tract. Phospholipid hydrolysis in the intestinal lumen can also be accomplished by group 1B phospholipase A_2 secreted by the pancreas, and triglyceride hydrolysis is usually attributed to pancreatic triglyceride lipase (PTL). Thus, it is difficult to know if CEL or these other enzymes plays the primary role in each digestive process or if CEL provides only supplemental or compensatory lipolytic capacity in the digestive tract. Mackay et al. have suggested that CEL works with phospholipase A_2 in promoting optimal intestinal cholesterol absorption from vesicular substrates (50). However, demonstrating the physiological significance of CEL in the absorption of any of these lipid nutrients is a difficult task.

Animal models have been utilized to explore the function of CEL in the digestive tract. Unfortunately, each model also has its unique set of advantages and limitations. The fact that the jury is still out on this issue despite years of research, which continues today, underscores the difficulties as well as the importance of such experiments. In this section, we begin with a brief overview of lipid digestion and absorption and chylomicron production. Thereafter, we present the evidence for and against a role for CEL in the absorption of each class of dietary lipids and end with our most recent findings and current understanding of how CEL may influence dietary lipid metabolism.

Overview

Lipid digestion begins in the stomach where the action of preduodenal lipase hydrolyzes up to 30% of ingested triglycerides into diglycerides and free fatty acids before delivery to the duodenum (51, 52). In the duodenum, partially digested lipid mixes with pancreatic and biliary secretions, which are essential to its further digestion and absorption. The pancreas produces an abundance of various lipases, including CEL, that together are capable of hydrolyzing all the dietary lipids to free fatty acids and glycerol, cholesterol, and glycerophosphocholine. The detergent properties of bile acids provide micelles for the solubilization of these lipids and their digestive products. The various digestive products transported in bile salt micelles are readily absorbed by enterocytes. The extent to which absorption is facilitated by specific transporters or occurs by diffusion is an ongoing debate. Discussion of this latter topic is beyond the scope of the current review.

In the enterocytes, triglycerides are resynthesized from fatty acids and monoglycerides or glycerol in the smooth endoplasmic reticulum (53, 54). Chylomicron assembly begins in the rough endoplasmic reticulum with the synthesis and initial lipidation of apolipoprotein B (apoB) (55). This process requires the participation of microsomal triglyceride transfer protein (56, 57). The core of these initial particles is enlarged by the addition of triglycerides and cholesteryl esters in the smooth endoplasmic reticulum. Prechylomicron particles are then transported from the endoplasmic reticulum to the *cis*-Golgi by an energy dependent mechanism that is not yet well understood (54). In the Golgi complex, the neutral lipid core is enlarged with additional triglyceride and cholesteryl esters. ApoA-IV, apoC-III, and apoA-I are also added to the surface as the particle enlarges. Secretory vesicles containing from one to several mature chylomicrons bud from the *trans*-Golgi and migrate to the basolateral regions of the enterocyte where they fuse with plasma membrane and release their contents into intestinal lacteals. Chylomicrons are transported by the intestinal lymph into the thoracic duct, which finally empties into the right subclavian vein. During circulation, core triglycerides are hydrolyzed by lipoprotein lipase in various tissues and the liberated fatty acids are used for immediate energy needs or are stored as triglycerides in adipose tissue. The chylomicron remnant particles acquire apoE in the circulation and are removed from the plasma by receptors on hepatocytes that recognize apoE (58).

The role of CEL in lipid digestion and absorption is implicated by its abundance in the gastrointestinal tract. This enzyme constitutes up to 5% of the protein in pancreatic juice (1, 2). The abundance of CEL along the intestinal tract decreases in a proximal to distal gradient with very small amount of CEL present in the terminal ileum (59, 60). The CEL present in the intestinal lumen is attached to the brush border of enterocytes via its heparin binding domain (61). There is a growing body of data indicating that pancreatic CEL may also be taken up by enterocytes as an intact protein. The latter observation was made originally by Gallo et al. (62) based on immunocytochemistry of rat intestine. These results have now been corroborated by others in later studies (63, 64). The CEL found intracellularly in intestine is most likely derived from pancreas since CEL is not expressed in the intestine (65, 66). A recent report suggested that the pancreatic CEL is taken up by enterocytes in association with the chaperone protein Grp94 through an endocytic pathway (66). In differentiated Int407 cells, the endocytosed CEL passed through the Golgi complex and was secreted as an intact protein through the basolateral membrane (64). Whether CEL is transcytosed through the basolateral side of the intestine and carried by the lymph into circulation has not been examined. Nevertheless, the location of CEL in the gastrointestinal tract is consistent with its participation in dietary lipid digestion and absorption, and potentially also in the intracellular processing of the digested lipids during chylomicron assembly and secretion. The evidence for and against each of these possible functions is discussed below.

Triglyceride absorption

The one function of CEL that is not disputed is that of milk-fat digestion in infants. As discussed previously, CEL is synthesized and secreted in abundance by lactating mammary glands of most mammalian species. In this regard, the CEL present in milk is probably more important than the CEL secreted by the pancreas in milk-fat digestion. It has been shown previously that CEL is not inactivated during passage through the stomach in the neonates (67–70), suggesting that this enzyme may be functional in the intestinal lumen and participates in lipid digestion prior to the maturation of the pancreas. Evidence in support of this hypothesis comes from both human and animal studies. In low birth-weight human infants, fat absorption increased up to 40% with concomitant increased weight gain when they were fed raw breast milk or formula supplemented with breast milk containing CEL as opposed to boiled milk or formula without breast milk supplementation (71, 72). Fat excretion also decreased by 50% in low birth-weight infants when fed the diets containing CEL. Kittens were also found to gain 50% less weight when fed a formula without CEL instead of nursed littermates or littermates fed CEL-supplemented formula (73). In our studies with CEL-null mice, we found that pups nursed by CEL-null dams contained large amounts of tri-, di-, and mono-glycerides in their colon contents while none was detectable in pups nursed by

control dams (74). Moreover, the lack of CEL in the digestive tract not only led to nutritional deficit in neonates, but evidence suggests that the undigested lipid is injurious to the intestinal epithelium of these mice, especially in the ileum (75–79). Taken together, these results suggest the importance of CEL in caring for premature and low birthweight infants, who typically have poor gastrointestinal function and are prone to inflammatory diseases of the bowel (80–83). The physiological significance for the lack of CEL in rat and cow milk has not been determined.

A role for CEL in triglyceride digestion and absorption in the adults is less clear. Although CEL is an abundant enzyme in the pancreatic juice of adults, PTL is believed to play the primary role in this process (84). Current studies with CEL-null mice suggested that CEL plays only a compensatory or supplementary role in triglyceride digestion (unpublished observations). It is important to note, however, that CEL may be important for digesting glycerolipids containing long chain polyenoic fatty acids. A number of laboratories have demonstrated that PTL has limited capacity to hydrolyze these lipids while CEL readily digests them to free fatty acids and glycerol (85–88). A direct test of the relative importance of CEL and PTL in digestion of specific glycerolipids as well as general triglyceride digestion and absorption awaits comparison of CEL- and PTL-knockout mice with respect to these functions.

An often ignored CEL activity is its ability to hydrolyze monoacylglycerols. While current dogma holds that monoacylglycerols are absorbed directly by the mucosa via passive diffusion, this tenet is based at least partly on the observation that PTL digests triglycerides only to fatty acids and 2-monoacylglycerol. CEL is required for the complete digestion of the glycerol lipids to fatty acids and glycerol (85–88). For adults, the physiological significance of this activity is unclear, but it is worth noting that approximately 40% of 2-monoacylglycerols are reduced to fatty acid plus glycerol prior to their absorption by the intestine (89). In neonates, digestion of the monoglycerols may be relatively important for absorption since pups nursed by CEL-null dams have significant amounts of monoacylglycerol in their fecal material (74). How the absence of this activity affects the rate of lipid absorption or the process of chylomicron assembly and secretion in adults remains to be determined.

Cholesterol absorption

The role of CEL in intestinal cholesterol absorption has been debated for nearly 40 years. Its importance in the digestion and absorption of cholesteryl esters was directly demonstrated several years ago by analysis of CEL knockout mice, which showed that intestinal absorption of dietary cholesteryl oleate was reduced by >60% in the absence of CEL (90). These results were later confirmed by another laboratory using independently generated CEL knockout mice (91). Both of these studies revealed that the lack of CEL has no influence on intestinal absorption of unesterified cholesterol from a single bolus meal (90, 91). Since both dietary cholesterol and cholesterol in the bile are predominantly unesterified, the importance of CEL in intestinal cholesterol absorption is not obvious. In a follow-up study, we used a lymph fistula model to compare the rate of cholesterol absorption between wild type and CEL-null mice. Results from these studies showed that the lack of CEL also had no influence on the rate of transport from the intestinal lumen to the lymph (19). Taken together, these results suggest that CEL in the digestive tract is not involved in regulating the amount or the rate of cholesterol absorbed by the intestine.

One must be circumspect, however, before casting aside a body of evidence that suggest a functional role of CEL with respect to cholesterol absorption. Were there differences in experimental conditions that can account for conflicting results? Is it also possible that the different conditions used in each experiment reflect different physiological states, thus yielding different information regarding the role of CEL in cholesterol absorption? In previous studies, cholesterol absorption was shown to be reduced by 80% when pancreas and bile diverted rats were supplied with bile and pancreatic juice from which CEL had been removed, in comparison to rats given bile and pancreatic juice containing normal amounts of the enzyme (92). These results were supported by studies in which the CEL-specific inhibitors WAY-121,751 and WAY-121,898 were shown to inhibit cholesterol absorption in both rats and dogs (93) and reduced serum cholesterol level in cholesterol-fed rats (94). In contrast to these results, Watt and Simmonds showed that intestinal cholesterol absorption has no requirement for pancreatic juice (95). It is possible that the CEL may be retained on the microvillus of intestine after pancreatic diversion and this may account for the difference between the latter studies with results of Gallo and her colleagues (92).

The in vitro cell culture studies also yielded controversial results regarding the role of CEL in cholesterol transport in intestinal cells. We have previously shown that CEL has direct cholesterol binding and transfer activities (96). This observation together with the well-documented heparin binding properties of CEL has led to the hypothesis that CEL may directly facilitate cholesterol uptake by cells at the cell surface (61). This hypothesis is consistent with results reported by Lopez-Candales et al. (48) showing a 4-fold increase in cholesterol uptake by Caco-2 cells when CEL was included in the incubation medium. However, our laboratory did not detect any effect of CEL on Caco-2 uptake of unesterified cholesterol, and our results showed that CEL only facilitated cellular uptake of esterified cholesterol (47). The results obtained by Shamir and colleagues were similar to those made in our laboratory (49).

The discrepancy between results of these studies were partially resolved by recent experiments examining the role of CEL in combination with phospholipase A_2 in intestinal cholesterol absorption. In these studies, absorption of micellar cholesterol by Caco-2 cells was inhibited by incorporation of phospholipid in the micelles (50, 97). The Ikeda study also showed that CEL facilitated cholesterol absorption in bile and pancreatic juice-diverted rats when the substrate was presented in a phospholipid-rich vesicle (97). A careful comparison of both the in vivo and

in vitro data suggested that the amount and physical state (lipid emulsion versus micelles versus vesicles) of the infused cholesterol significantly impacts the apparent contribution of CEL in cholesterol absorption. The sum of all the available data to date indicates that CEL has no direct role in absorption of unesterified cholesterol under normal conditions. However, this enzyme serves an important compensatory or supplementary role in providing sufficient lipolytic enzyme activities for complete triglyceride and phospholipid hydrolysis, which are required for optimal absorption of cholesterol (50, 98).

Phospholipid digestion and absorption

Phospholipid hydrolysis in the intestinal lumen is thought to be mediated primarily by the group 1B phospholipase A_2 secreted by the pancreas (84). Since CEL also has avid phospholipase activity (1), this enzyme may also participate in phospholipid digestion in the intestinal lumen. As discussed above, Ikeda et al. (97) showed that CEL facilitated cholesterol absorption in bile and pancreatic juice-diverted rats when the substrate was presented in a phospholipid-rich vesicle. Their study revealed that the mechanism is related to CEL-catalyzed phospholipid hydrolysis. An important clue demonstrating the presence of alternative enzyme(s), other than the group 1B phospholipase A_2, capable of phospholipid digestion in the intestinal lumen was obtained by comparing dietary phospholipid hydrolysis and absorption between wild type and phospholipase A_2 knockout mice. These studies revealed that phospholipid digestion and intestinal absorption was similar between animals with or without a functional group 1B phospholipase A_2 gene (99). Whereas other intestinal phospholipases, particularly the intestinal phospholipase B (100–102), may account for the intestinal phospholipase activity in the absence of the pancreatic phospholipase A_2, expression of the phospholipase B enzyme is limited to the distal small intestine and one would expect a delayed phospholipid absorption if this is the compensatory enzyme for phospholipid hydrolysis. The fact that no delay in phospholipid absorption was observed in the phospholipase A_2-knockout mice is consistent with the hypothesis that the phospholipase activity of CEL, which is present in the proximal intestine, is sufficient to facilitate phospholipid digestion and absorption in the absence of phospholipase A_2.

In addition to a possible role for CEL as a compensatory enzyme for phospholipase A_2 in intestinal phospholipid digestion, the lysophospholipase activity of CEL may also be important in limiting the concentration of this phospholipid digestive product in the intestinal lumen. It is interesting to note that the activity of phospholipase A_2 is usually elevated in the intestinal epithelia of patients with inflammatory bowel disease. Importantly, the increased bacterial translocation and decreased transepithelial electrical resistance in the intestine of these patients are attributed to elevated concentration of lysophospholipids (103). Since CEL is the only enzyme secreted by the pancreas that is capable of lysophospholipid hydrolysis in the intestinal lumen (1), this protein may function to protect the intestinal epithelia through hydrolysis of lysophospholipids. Additional studies are warranted to test this hypothesis.

Vitamin absorption

The importance of CEL in facilitating intestinal absorption of fat-soluble vitamins, especially vitamins A and E, has also been debated. Most dietary vitamin E is in the form of tocopherol that does not require any lipolytic processing before absorption. Vitamin A, however, is often esterified in natural diets and in dietary supplements as retinyl palmitate and retinyl acetate, respectively, and must be hydrolyzed to fatty acid and retinol prior to absorption. For nursing infants, the primary source of vitamin A is the retinyl palmitate in breast milk. Since CEL is the major lipase in neonates (see discussion above), this enzyme serves a primary role in neonatal vitamin absorption. As shown in mouse pups nursed by CEL-null dams, hepatic stores of vitamin A were reduced by ~50% in the absence of milk CEL as compared to pups nursed by control dams (104). This deficit persisted into the second week of life until the pancreas begins to secrete sufficient amounts of endogenous lipase for digestion of milkfat and the associated retinyl ester.

The role of CEL in vitamin absorption in adults is less clear. The possible importance of CEL was first suggested by Fernandez and Borgstrom (105), who showed that tetrahydrolipstatin dramatically reduced retinyl ester absorption in adult rats even when the vitamin was supplied in mixed micelles with bile salts, free fatty acids, and monoacylglycerol. Since tetrahydrolipstatin inhibits PTL as well as CEL, these results did not conclusively demonstrate the participation of CEL in vitamin absorption. In fact, earlier studies suggested that the PTL has retinyl ester hydrolytic activity (106). A third enzyme with the potential to contribute to vitamin A ester digestion and absorption is the intestinal phospholipase B. Rigtrup and Ong demonstrated that both CEL and phospholipase B are potent retinyl ester hydrolases and can be distinguished by their location either proximally or distally in the intestine, respectively (59, 60). Our own studies with CEL knockout mice indicated that CEL contributes ~50% of the retinyl ester hydrolytic activity in pancreatic extracts (104). Thus, any one of these three enzymes can potentially facilitate sufficient vitamin A absorption to maintain normal circulating levels and hepatic stores. One study with CEL knockout mice showed no difference in retinyl palmitate absorption between control and CEL-null mice (91), whereas another study showed that CEL-null mice absorbed 40% less retinyl palmitate than their control counterparts (104). Thus, as with digestion and absorption of other lipid nutrients, it is likely that CEL provides supplementary or compensatory activity in vitamin absorption in adults.

Chylomicron assembly and secretion

Aside from serving a compensatory or supplementary role to other lipolytic enzymes in the digestive tract, the physiological importance of CEL in lipid absorption re-

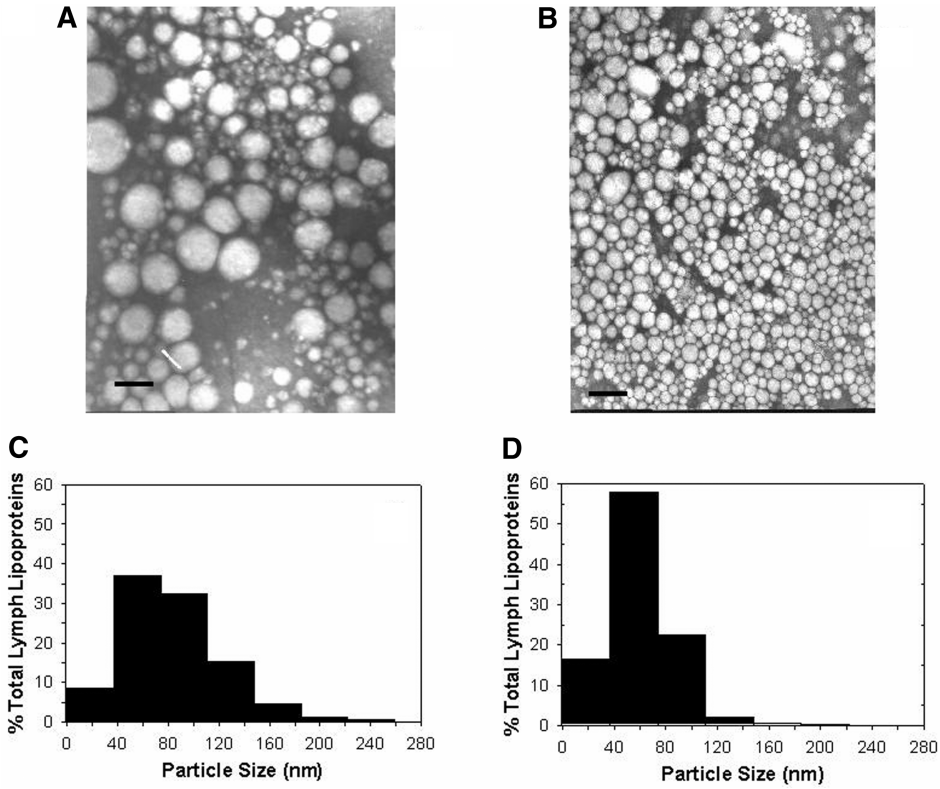

Fig. 2. Size distribution of lymph lipoproteins from control and CEL-null mice. Lymph was collected from $CEL^{+/+}$ (A and C) and $CEL^{-/-}$ (B and D) lymph fistula mice 4 h after the onset of duodenal lipid infusion. The samples were adhered to 300-mesh formvar grids, and stained with either 1% phosphotungstic acid, pH 6.9 (A), or dual stained with 4% osmium tetroxide and 1% phosphotungstic acid in 0.1% sucrose (B). Magnification = 40,000× with the bars indicating 200 nm in length. Approximately 300–400 particles were counted from representative micrographs and collective results are shown for lipoprotein particle size in $CEL^{+/+}$ (C) and $CEL^{-/-}$ (D) mice. (Reproduced from Reference 19, and used by permission of the American Society for Biochemistry and Molecular Biology.)

mains poorly understood. Accordingly, our laboratories have recently focused on another enzymatic property of CEL, i.e., its lipoamidase activity and its ability to hydrolysis ceramide (17). This CEL activity is unique among proteins secreted by the pancreas and found in the intestinal lumen. Thus, the ceramide hydrolytic activity of CEL may play a significant role in lipid nutrient transport. To examine this possibility, we have compared chylomicron production and secretion in wild-type and CEL knockout mice. Consistent with results observed previously, neither the amount nor the rate of cholesterol and triglyceride absorption was affected by the presence or absence of CEL in the digestive tract. However, when the lipoproteins secreted into lymph of control and CEL-null mice were examined, a dramatic difference in lipoprotein particle sizes was observed (19). Whereas the majority of the lipoproteins secreted by control mice after lipid infusion are the size of chylomicron particles (>80 nm in diameter), the majority of lipoprotein particles in the intestinal lymph of CEL knockout mice were of the size of VLDL, ranging between 30–80 nm in diameter (**Fig. 2**). The decrease in lipoprotein particle size was related to decreased ceramidase activity in the proximal intestine of CEL-null mice in comparison to wild type control animals (19).

A direct relationship between the ceramidase activity of CEL and its ability to modulate intestinal lipoprotein biosynthesis was demonstrated in vitro with Caco-2 cells. When differentiated Caco-2 cells were cultured on transwell membranes and cholesterol transport from micellar substrates in the apical compartment to lipoproteins secreted into the basolateral compartment was measured, the inclusion of CEL in the apical medium increased cholesterol secretion associated with chylomicron-sized particles in the basolateral compartment (19). The incubation of sphingomyelinase, which produces ceramide, instead of CEL, which hydrolyzes ceramide, resulted in inhibition of chylomicron assembly and secretion. The latter results were consistent with those reported previously (107, 108). Interestingly, the inclusion of CEL in the incubation medium along with sphingomyelinase resulted in optimal production of chylomicron-sized lipoproteins by the Caco-2 cells (19). Thus, these results provided additional support to the hypothesis that the ceramidase activity of CEL plays an important role in intestinal lipoprotein assembly and transport. However, whether CEL acts intracellularly after

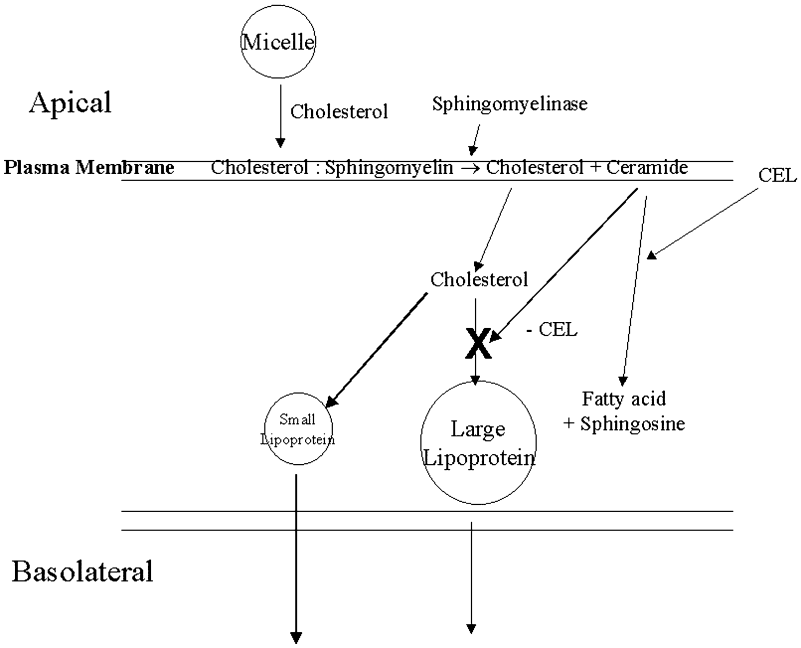

Fig. 3. Schematic diagram depicting the proposed participation of SMase and CEL in cholesterol transport in enterocytes. This diagram shows that the initial step of cholesterol absorption is the intercalation of the micellar cholesterol from the lumen to the apical membrane of enterocytes. The second step of cholesterol transport from the plasma membrane to the cell interior requires the hydrolysis of the sphingomyelin by SMase present in the intestinal lumen or in the cell interior. Ceramide generated as a result of sphingomyelin hydrolysis can then be hydrolyzed by CEL present in either the lumen (or membrane bound) or CEL endocytosed into the cells. Lack of ceramide hydrolysis will result in blockage of large lipoprotein assembly and secretion by the intestinal cells. (Reproduced from Reference 19, and used by permission of the American Society for Biochemistry and Molecular Biology.)

its endocytosis into the intracellular compartment or acts by mediating lipid transport in the brush border membranes remains unaddressed.

Our current view of how CEL affects chylomicron assembly and secretion is shown schematically in **Fig. 3**. During lipid absorption, micellar cholesterol intercalates into the apical membrane of enterocytes (109–111). The membrane-bound cholesterol is subsequently imported into the cell interior where lipoprotein assembly occurs. However, this second step of cholesterol uptake requires the hydrolysis of membrane sphingomyelin by sphingomyelinase (112, 113), which in turn generates free fatty acid and ceramide. Naturally occurring long chain ceramides generated through sphingomyelin hydrolysis are potential promoters of Golgi disassembly and are capable of disrupting protein trafficking through intracellular secretory pathways (114, 115). Thus in the absence of CEL, the accumulation of ceramide in the enterocytes may disrupt normal lipid and protein trafficking pathways and result in abnormal smaller-sized lipoproteins being produced. The role of CEL in ceramide hydrolysis may be important to relieve ceramide disruption of intracellular trafficking pathways, thereby promoting proper delivery of the exogenous lipids to intracellular locations where normal chylomicron assembly can occur. Additional supportive data are necessary to confirm this hypothesis.

LIPOPROTEIN METABOLISM AND ATHEROSCLEROSIS

One area of investigation that has not been explored extensively is the possible role of CEL in plasma lipoprotein metabolism and atherosclerosis. In addition to its presence in the digestive tract, CEL is synthesized in liver (4, 7, 65), macrophages (9, 46), endothelial cells (10), and heart (116). CEL protein and enzyme activity are also found in the circulation (117–119). However, its function in these settings has not been studied extensively in terms of its role in lipoprotein metabolism and cardiovascular disease. Since bile salt is required for enzyme activation, it has long been thought that this enzyme has minimal activity outside the digestive tract where bile salt concentration is low. However, it must be noted that fasting serum bile salt concentration is approximately 10 μM and increases by 3-fold postprandially (120, 121). Bile acid concentrations are also typically 6-fold higher in portal blood than in peripheral circulation and may reach as high as 100 μM (122). Since the K_d for bile salt binding to CEL is approximately 20 μM and enzyme activation can easily be accomplished at 10-μM cholate (1), circulating bile acid and bile acid in portal blood is sufficient for CEL activation. Thus, CEL may have systemic functions that have not been appreciated previously. This section summarizes the current

information available on possible function of this enzyme in lipoprotein metabolism.

Hepatic lipoprotein metabolism

There are at least two neutral cholesteryl ester hydrolases in the liver. One of these enzymes is primarily cytosolic and is immunologically and biochemically distinct from CEL (123–126). The identity of a second cytosolic cholesterol esterase as being CEL was first established by Camulli et al. (6), who purified the protein from rat liver and showed that it was identical to the pancreatic enzyme based on immunoreactivity with CEL-specific antibodies, bile salt activation, and N-terminal amino acid sequencing. These results were subsequently confirmed by RNA analysis (4, 7, 65). The hepatic CEL was found to be present in specific endosomal compartments of hepatocytes (127). In a separate study, Winkler et al. (128) showed that the hepatic-derived CEL can be secreted directly by liver cells. Thus, the CEL found in the endosomal compartment of the liver may be recaptured enzyme secreted by hepatic cells. Hepatic secretion of CEL and its presence on liver cell surface and in endosomal compartments suggest that this enzyme may play a role in the hepatic secretion-capture pathway by which lipoproteins are metabolized (58).

A role for CEL in hepatic lipoprotein metabolism was suggested earlier by our observation that inclusion of CEL in the incubation medium facilitated the selective uptake of cholesteryl esters in HDL by liver cells (129). The increase in selective uptake of HDL-derived cholesteryl esters induced by CEL is specific for this enzyme as the presence of antibodies against CEL resulted in inhibition of the selective uptake process (129). Recently, in addition to its presence in endosomal compartments (127), CEL was also shown to be present in lipid rafts and to co-fractionate with the HDL receptor, SR-BI, in liver cells (130). These results suggest that CEL participates directly in the selective uptake of HDL-cholesteryl esters. Since cholesterol from the selective uptake process is thought to be shunted primarily to the bile, CEL may contribute to cholesterol homeostasis in the liver and the balance of biliary cholesterol and bile acids. Analysis of CEL knockout mice with respect to these parameters is underway to test this possibility.

Lipid metabolism in the vasculature

Carboxyl ester lipase is also present in the vessel wall (10, 119, 131, 132). This vascular enzyme mostly originates in endothelial cells (10). The CEL is also synthesized in human macrophages in a manner that is inducible by oxidized LDL (9). This latter observation suggested that CEL in the vessel wall participates in the atherosclerotic process. Whether CEL protects or contributes to atherosclerosis has not been studied to date. Although the precise function of CEL in the vessel wall remains speculative, some aspects of atherogenesis suggest a possible protective role for CEL. It is important to note that one recognized major contributor for atherosclerosis plaque development is oxidized LDL (133, 134). In addition to its role in macrophage foam cell formation, oxidized LDL also contributes directly to other aspects of atherogenesis. These include the induction of endothelial cell damage, increased adhesion of monocytes, and proliferation of macrophages and smooth muscle cells. Although the precise mechanisms leading to these atherosclerotic events are not fully understood at the present time, current data suggest that lysophosphatidylcholine (lysoPC), a by-product of phospholipid hydrolysis generated during LDL oxidation, may play a key role. For example, the level of lysoPC in atherosclerotic lesions was reported to be elevated 800% in comparison with lesion-free areas of the aorta (135). In in vitro cell culture experiments, lysoPC has been shown to impair endothelium-dependent arterial relaxation (136) to act as a chemoattractant for monocytes (137), to induce monocyte adhesion to arterial endothelial cells (138), and to promote macrophage cell proliferation (139). The production of lysoPC in atherosclerotic plaque areas was attributed to the activation of intrinsic phospholipase A_2 activity in LDL during oxidation (140). In addition, induction of Type II phospholipase A_2 secretion by arterial cells during the inflammatory process may also be responsible for the increased lysoPC content in the lesion areas (141–143). A role of phospholipase A_2 and its hydrolytic products in promotion of atherogenesis is further supported by recent observations of increased fatty streak lesions in the aorta of phospholipase A_2-transgenic mice (144, 145). Taken together, these observations suggest that the expression and presence of CEL in the vessel wall may be a protective mechanism against atherosclerosis by reducing lysoPC level in the vasculature.

Another class of lipid signaling molecules important for the modulation of vascular cell functions is the sphingolipids, including sphingosine 1-phosphate and ceramide. Lipoproteins retained in the extracellular matrix of the arterial wall may be hydrolyzed by sphingomyelinase, resulting in LDL aggregation and retention (146). The hydrolysis of sphingolipids in LDL also resulted in the accumulation of 10–50-fold excess amounts of ceramide in the vessel wall (146). Ceramide is another atherogenic lipid signaling molecule present in oxidized LDL that has previously been shown to promote vascular smooth muscle cell proliferation (147, 148). A direct contributory role of ceramide in vascular occlusive disease was also supported by experiments showing that oxidative stress-induced vascular changes are related to redox stress-induced proliferation of smooth muscle cells through ceramide-induction of the mitogen activated protein kinase pathway (149). Additionally, lactosylceramide has been shown to mediate tumor necrosis factor induced nuclear factor-κB expression and expression of ICAM-1 in vascular endothelial cells (150). Lactosylceramide also stimulates the expression of adhesion molecules such as CD11/CD8 in neutrophils. Taken together, these results suggest that ceramide and its homologs contribute to the initiation and the progression of atherosclerosis. Accordingly, the expression and presence of CEL in the vessel wall may be a protective mechanism to reduce the level of these atherogenic lipid signaling molecules.

The potential role of vascular CEL in protection against oxidized LDL induced atherosclerosis has not been tested vigorously in animal models because, unlike in humans, neither mouse macrophages nor endothelial cells produce CEL (9, 10). However, CEL decreased the lysoPC content in oxidized LDL (119) as well as decreased the cytotoxic effects of lysoPC in cell culture experiments (10). Direct demonstration of the vascular protective role of CEL awaits the generation of vascular-specific CEL transgenic mice and the determination of their susceptibility to atherosclerosis.

CEL in circulation

The presence of low amounts of CEL in the circulation is well documented in the literature (117–119). This enzyme may be derived from the liver, circulating monocytes and eosinophils, or the pancreas after its transcytosis through the intestine. The physiological function of the CEL circulating in plasma is also not clear. However, there is increasing evidence that the circulating CEL may also participate in lipoprotein metabolism and atherosclerosis. As discussed previously, CEL reduces the atherogenic potential of oxidized LDL by hydrolyzing the excessive lsoPC generated during the lipoprotein oxidation process. We have also reported a positive correlation between plasma CEL level and LDL cholesterol (117), suggesting that CEL may play a role in LDL metabolism in humans. In support of this hypothesis was the observation that CEL facilitates the conversion of the larger and less atherogenic LDL to smaller LDL particles in vitro (117). In this regard, the CEL in circulation may be pro-atherogenic. In contrast, CEL has also been shown to promote selective uptake of HDL-associated cholesteryl esters by liver cells (129). In this regard, CEL may be anti-atherogenic by facilitating reverse cholesterol transport and reducing cholesterol accumulation in peripheral tissues. Thus, additional studies are warranted to definitively identify the role of circulating CEL in lipoprotein metabolism. Information obtained from such studies may provide additional insights and alternative strategies for treatment of hyperlipidemia to reduce the incidence of hyperlipidemia-related diseases.

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REFERENCES

1. Rudd, E. A., and H. L. Brockman. 1984. Pancreatic carboxyl ester lipase (cholesterol esterase). *In* Lipases. B. Borgstrom and H. L. Brockman, editors. Elsevier Science, New York, NY. 185–204.
2. Wang, C. S., and J. A. Hartsuck. 1993. Bile salt-activated lipase: a multiple function lipolytic enzyme. *Biochim. Biophys. Acta.* **1166:** 1–19.
3. Hui, D. Y. 1996. Molecular biology of enzymes involved with cholesterol ester hydrolysis in mammalian tissues. *Biochim. Biophys. Acta.* **1303:** 1–14.
4. Zolfaghari, R., E. H. Harrison, A. C. Ross, and E. A. Fisher. 1989. Expression in Xenopus oocytes of rat liver mRNA coding for a bile salt-dependent cholesteryl ester hydrolase. *Proc. Natl. Acad. Sci. USA.* **86:** 6913–6916.
5. Zolfaghari, R., E. H. Harrison, J. H. Han, W. J. Rutter, and E. A. Fisher. 1992. Tissue and species differences in bile salt-dependent neutral cholesteryl ester hydrolase activity and gene expression. *Arterioscler. Thromb.* **12:** 295–301.
6. Camulli, E. A., M. J. Linke, H. L. Brockman, and D. Y. Hui. 1989. Identity of a cytosolic neutral cholesterol esterase in rat liver with the pancreatic bile salt stimulated cholesterol esterase. *Biochim. Biophys. Acta.* **1005:** 177–182.
7. Verine, A., N. Bruneau, A. Valette, J. Le Petit-Thevenin, E. Pasqualini, and D. Lombardo. 1999. Immunodetection and molecular cloning of a bile salt dependent lipase isoform in HepG2 cells. *Biochem. J.* **342:** 179–187.
8. Holtsberg, F. W., L. E. Ozgur, D. E. Garsetti, J. Myers, R. W. Egan, and M. A. Clark. 1995. Presence in human eosinophils of a lysophospholipase similar to that found in the pancreas. *Biochem. J.* **309:** 141–144.
9. Li, F., and D. Y. Hui. 1997. Modified low density lipoprotein enhances the secretion of bile salt-stimulated cholesterol esterase by human monocyte-macrophages. Species-specific difference in macrophage cholesteryl ester hydrola. *J. Biol. Chem.* **272:** 28666–28671.
10. Li, F., and D. Y. Hui. 1998. Synthesis and secretion of the pancreatic-type carboxyl ester lipase by human endothelial cells. *Biochem. J.* **329:** 675–679.
11. Lombardo, D. 2001. Bile salt-dependent lipase: its pathophysiological implications. *Biochim. Biophys. Acta.* **1533:** 1–28.
12. DiPersio, L. P., R. N. Fontaine, and D. Y. Hui. 1990. Identification of the active site serine in pancreatic cholesterol esterase by chemical modification and site specific mutagenesis. *J. Biol. Chem.* **265:** 16801–16806.
13. DiPersio, L. P., R. N. Fontaine, and D. Y. Hui. 1991. Site-specific mutagenesis of an essential histidine residue in pancreatic cholesterol esterase. *J. Biol. Chem.* **266:** 4033–4036.
14. Stout, J. S., L. D. Sutton, and D. M. Quinn. 1985. Acylenzyme mechanism and solvent isotope effects for cholesterol esterase-catalyzed hydrolysis of p-nitrophenyl butyrate. *Biochim. Biophys. Acta.* **837:** 6–12.
15. Feaster, S. R., D. M. Quinn, and B. L. Barnett. 1997. Molecular modeling of the structures of human and rat pancreatic cholesterol esterases. *Protein Sci.* **6:** 73–79.
16. DiPersio, L. P., and D. Y. Hui. 1993. Aspartic acid 320 is required for optimal activity of rat pancreatic cholesterol esterase. *J. Biol. Chem.* **268:** 300–304.
17. Hui, D. Y., K. Hayakawa, and J. Oizumi. 1993. Lipoamidase activity in normal and mutagenized pancreatic cholesterol esterase (bile salt-stimulated lipase). *Biochem. J.* **291:** 65–69.
18. Nyberg, L., A. Farooqi, L. Blackberg, R. D. Duan, A. Nilsson, and O. Hernell. 1998. Digestion of ceramide by human milk bile salt stimulated lipase. *J. Pediatr. Gastroenterol. Nutr.* **27:** 560–567.
19. Kirby, R. J., S. Zheng, P. Tso, P. N. Howles, and D. Y. Hui. 2002. Bile salt-stimulated carboxyl ester lipase influences lipoprotein assembly and secretion in intestine. A process mediated via ceramide hydrolysis. *J. Biol. Chem.* **277:** 4104–4109.
20. Wang, X., C. S. Wang, J. Tang, F. Dyda, and X. C. Zhang. 1997. The crystal structure of bovine bile salt activated lipase: insights into the bile salt activation mechanism. *Structure.* **5:** 1209–1218.
21. Chen, J. C. H., L. J. W. Miercke, J. Krucinski, J. R. Starr, G. Saenz, X. Wang, C. A. Spilburg, L. G. Lange, J. L. Ellsworth, and R. M. Stroud. 1998. Structure of bovine pancreatic cholesterol esterase at 1.6 angstrom: Novel structural features involved in lipase activation. *Biochemistry.* **37:** 5107–5117.
22. Moore, S. A., R. L. Kingston, K. M. Loomes, O. Hernell, L. Blackberg, H. M. Baker, and E. N. Baker. 2001. The structure of truncated recombinant human bile salt-stimulated lipase reveals bile salt-independent conformational flexibility at the active site loop and provides insights into heparin binding. *J. Mol. Biol.* **312:** 511–523.
23. Cygler, M., and J. D. Schrag. 1997. Structure as a basis for understanding interfacial properties of lipases. *Methods Enzymol.* **284:** 3–27.
24. Blackberg, L., D. Lombardo, O. Hernell, O. Guy, and T. Olivecrona. 1981. Bile salt-stimulated lipase in human milk and carboxyl ester hydrolase in pancreatic juice. *FEBS Lett.* **136:** 284–288.
25. Lombardo, D., D. Campese, L. Multigner, H. LaFont, and A. DeCaro. 1983. On the probable involvement of arginine residues in the bile salt binding site of human pancreatic carboxylic ester hydrolase. *Eur. J. Biochem.* **133:** 327–333.
26. Blackberg, L., and O. Hernell. 1993. Bile salt-stimulated lipase in human milk. Evidence that bile salt induces lipid binding and activation via binding to different sites. *FEBS Lett.* **323:** 207–210.

27. Gjellesvik, D. R., J. B. Lorens, and R. Male. 1994. Pancreatic carboxylester lipase from Atlantic salmon (Salmo salar). cDNA sequence and computer-assisted modelling of tertiary structure. *Eur. J. Biochem.* **226:** 603–612.
28. Liang, Y., R. Medhekar, H. L. Brockman, D. M. Quinn, and D. Y. Hui. 2000. Importance of arginines 63 and 423 in modulating the bile salt-dependent and bile salt-independent hydrolytic activities of rat carboxyl ester lipase. *J. Biol. Chem.* **275:** 24040–24046.
29. Jacobson, P. W., P. W. Wiesenfeld, L. L. Gallo, R. L. Tate, and J. C. Osborne. 1990. Sodium cholate-induced changes in the conformation and activity of rat pancreatic cholesterol esterase. *J. Biol. Chem.* **265:** 515–521.
30. Hui, D. Y., and J. A. Kissel. 1990. Sequence identity between human pancreatic cholesterol esterase and bile salt-stimulated milk lipase. *FEBS Lett.* **276:** 131–134.
31. Stromqvist, M., O. Hernell, L. Hansson, K. Lindgren, A. Skytt, L. Lundberg, A. S. Lidmer, and L. Blackberg. 1997. Naturally occurring variants of human milk bile salt-stimulated lipase. *Arch. Biochem. Biophys.* **347:** 30–36.
32. Lindquist, S., L. Blackberg, and O. Hernell. 2002. Human bile salt-stimulated lipase has a high frequency of size variation due to a hypervariable region in exon 11. *Eur. J. Biochem.* **269:** 759–767.
33. Bruneau, N., A. Nganga, E. A. Fisher, and D. Lombardo. 1997. O-glycosylation of C-terminal tandem repeated sequences regulates the secretion of rat pancreatic bile salt dependent lipase. *J. Biol. Chem.* **272:** 27353–27361.
34. Taylor, A. K., J. L. Zambaux, I. Klisak, T. Mohandas, R. S. Sparkes, M. C. Schotz, and A. J. Lusis. 1991. Carboxyl ester lipase: A highly polymorphic locus on human chromosome 9qter. *Genomics.* **10:** 425–431.
35. Kumar, V. B., T. Sasser, J. B. Mandava, H. Al Sadi, and C. Spilburg. 1997. Identification of 5′-flanking sequences that affect human pancreatic cholesterol esterase gene expression. *Biochem. Cell Biol.* **75:** 247–254.
36. Cox, D. G., C. K. T. Leung, E. M. Kyger, C. A. Spilburg, and L. G. Lange. 1990. Pancreatic cholesterol esterases. 1. Pancreatic cholesterol esterase induction during maturation. *Biochemistry.* **29:** 3842–3848.
37. Hernell, O., and T. Olivecrona. 1974. Human milk lipases. II. Bile salt stimulated lipase. *Biochim. Biophys. Acta.* **369:** 234–244.
38. Nilsson, J., L. Blackberg, P. Carlsson, S. Enerback, O. Hernell, and G. Bjursell. 1990. cDNA cloning of human milk bile salt stimulated lipase and evidence for its identity to pancreatic carboxylic ester hydrolase. *Eur. J. Biochem.* **192:** 543–550.
39. Baba, T., D. Downs, K. W. Jackson, J. Tang, and C. S. Wang. 1991. Structure of human milk bile salt activated lipase. *Biochemistry.* **30:** 500–510.
40. Lidmer, A. S., M. Kannius, L. Lundberg, G. Bjursell, and J. Nilsson. 1995. Molecular cloning and characterization of the mouse carboxyl ester lipase gene and evidence for expression in the lactating mammary gland. *Genomics.* **29:** 115–122.
41. Kannius-Janson, M., U. Lidberg, K. Hulten, A. Gritli-Linde, G. Bjursell, and J. Nilsson. 1998. Studies of the regulation of the mouse carboxyl ester lipase gene in mammary gland. *Biochem. J.* **336:** 577–585.
42. Lidberg, U., M. Kannius-Janson, J. Nilsson, and G. Bjursell. 1998. Transcriptional regulation of the human carboxyl ester lipase gene in exocrine pancreas. Evidence for a unique tissue-specific enhancer. *J. Biol. Chem.* **273:** 31417–31426.
43. Lidberg, U., J. Nilsson, K. Stromberg, G. Stenman, P. Sahlin, S. Enerback, and G. Bjursell. 1992. Genomic organization, sequence analysis, and chromosomal localization of the human carboxyl ester lipase (CEL) gene and a CEL-like (CELL) gene. *Genomics.* **13:** 630–640.
44. Qasba, M., and S. K. Safaya. 1984. Similarity of the nucleotide sequences of rat alpha-lactalbumin and the chicken lysozyme genes. *Nature.* **308:** 377–380.
45. Yu-Lee, L., L. Richter-Mann, C. Couch, F. Stewart, G. Mackinlay, and J. Rosen. 1986. Evolution of the casein multigene family: Conserved sequences in the 5′ flanking and exon regions. *Nucleic Acids Res.* **14:** 1883–1902.
46. Bengtsson, S. H. M., K. Madeyski-Bengtson, J. Nilsson, and G. Bjursell. 2002. Transcriptional regulation of the human carboxyl ester lipase gene in THP-1 monocytes: an E-box required for activation binds upstream stimulatory factors 1 and 2. *Biochem. J.* **365:** 481–488.
47. Huang, Y., and D. Y. Hui. 1990. Metabolic fate of pancreas-derived cholesterol esterase in intestine. An in vitro study using Caco-2 cells. *J. Lipid Res.* **31:** 2029–2037.
48. Lopez-Candales, A., M. S. Bosner, C. A. Spilburg, and L. G. Lange. 1993. Cholesterol transport function of pancreatic cholesterol esterase: Directed sterol uptake and esterification in enterocytes. *Biochemistry.* **32:** 12085–12089.
49. Shamir, R., W. J. Johnson, R. Zolfaghari, H. S. Lee, and E. A. Fisher. 1995. Role of bile salt-dependent cholesteryl ester hydrolase in the uptake of micellar cholesterol by intestinal cells. *Biochemistry.* **34:** 6351–6358.
50. Mackay, K., J. R. Starr, R. M. Lawn, and J. L. Ellsworth. 1997. Phosphatidylcholine hydrolysis is required for pancreatic cholesterol esterase- and phospholipase A2-facilitated cholesterol uptake into intestinal Caco-2 cells. *J. Biol. Chem.* **272:** 13380–13389.
51. Hamosh, M., and R. O. Scow. 1973. Lingual lipase and its role in the digestion of dietary lipid. *J. Clin. Invest.* **52:** 88–95.
52. Iverson, S. J., C. L. Kirk, M. Manosh, and J. Newsome. 1991. Milk lipid digestion in the neonatal dog: the combined actions of gastric and bile salt stimulated lipase. *Biochim. Biophys. Acta.* **1083:** 109–119.
53. Cartwright, I. J., D. Plonne, and J. A. Higgins. 2000. Intracellular events in the assembly of chylomicrons in rabbit enterocytes. *J. Lipid Res.* **41:** 1728–1739.
54. Mansbach, C. M., and P. Nevin. 1998. Intracellular movement of triacylglycerols in the intestine. *J. Lipid Res.* **39:** 963–968.
55. Glickman, R. M., J. N. Glickman, A. Magun, and M. Brin. 1991. Apolipoprotein synthesis in normal and abetalipoproteinemic intestinal mucosa. *Gastroenterology.* **101:** 749–755.
56. Wetterau, J. R., L. P. Aggerbeck, M. E. Bouma, C. Eisenberg, A. Munck, M. Hermier, J. Schmitz, G. Gay, D. J. Rader, and R. E. Gregg. 1992. Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. *Science.* **258:** 999–1001.
57. Wetterau, J. R., M. C. M. Lin, and H. Jamil. 1997. Microsomal triglyceride transfer protein. *Biochim. Biophys. Acta.* **1345:** 136–150.
58. Mahley, R. W., and Z. S. Ji. 1999. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *J. Lipid Res.* **40:** 1–16.
59. Rigtrup, K. M., and D. E. Ong. 1992. A retinyl ester hydrolase activity intrinsic to the brush border membrane of rat small intestine. *Biochemistry.* **31:** 2920–2926.
60. Rigtrup, K. M., L. R. McEwen, H. M. Said, and D. E. Ong. 1994. Retinyl ester hydrolytic activity associated with human intestinal brush border membranes. *Am. J. Clin. Nut.* **60:** 111–116.
61. Bosner, M. S., T. Gulick, D. J. S. Riley, C. A. Spilburg, and L. G. Lange. 1988. Receptor-like function of heparin in the binding and uptake of neutral lipids. *Proc. Natl. Acad. Sci. USA.* **85:** 7438–7442.
62. Gallo, L. L., Y. Chiang, G. V. Vahouny, and C. R. Treadwell. 1980. Localization and origin of rat intestinal cholesterol esterase determined by immunocytochemistry. *J. Lipid Res.* **21:** 537–545.
63. Bruneau, N., D. Lombardo, and M. Bendayan. 2000. The affinity binding sites of pancreatic bile salt-dependent lipase in pancreatic and intestinal tissues. *J. Histochem. Cytochem.* **48:** 267–276.
64. Bruneau, N., A. Nganga, M. Bendayan, and D. Lombardo. 2001. Transcytosis of pancreatic bile salt-dependent lipase through human Int407 intestinal cells. *Exp. Cell Res.* **271:** 94–108.
65. Kissel, J. A., R. N. Fontaine, C. Turck, H. L. Brockman, and D. Y. Hui. 1989. Molecular cloning and expression of cDNA for rat pancreatic cholesterol esterase. *Biochim. Biophys. Acta.* **1006:** 227–236.
66. Bruneau, N., D. Lombardo, and M. Bendayan. 1998. Participation of GRP94-related protein in secretion of pancreatic bile salt-dependent lipase and in its internalization by the intestinal epithelium. *J. Cell Sci.* **111:** 2665–2679.
67. Zoppi, G., G. Andreotti, F. Pajno-Ferrara, D. M. Njai, and D. Gaburro. 1972. Exocrine pancreas function in premature and full term neonates. *Pediatr. Res.* **6:** 880–886.
68. Lee, P. C., and E. Lebenthal. 1993. Prenatal and postnatal development of the human exocrine pancreas. *In* The Pancreas: Biology, Pathobiology, and Disease. V. Liang and W. Go, editors. Raven Press, New York, NY. 57–173.
69. Fredrikzon, B., and T. Olivecrona. 1978. Decrease of lipase and esterase activities in intestinal contents of newborn infants during test meals. *Pediatr. Res.* **12:** 631–634.
70. Lebenthal, E., and P. C. Lee. 1980. Development of functional responses in human exocrine pancreas. *Pediatrics.* **66:** 556–560.
71. Williamson, S., E. Finucane, H. Ellis, and H. R. Gamsu. 1978. Effect of heat treatment of human milk on absorption of nitrogen,

fat, sodium, and phosphorus by preterm infants. *Arch. Dis. Child.* **53:** 555–563.
72. Alemi, B., M. Hamosh, J. W. Scanlon, C. Salzman-Mann, and P. Hamosh. 1981. Fat digestion in very low birth-weight infants: effect of addition of human milk to low birth weight formula. *Pediatrics.* **68:** 484–489.
73. Wang, C. S., M. E. Martindale, M. M. King, and J. Tang. 1989. Bile-salt-activated lipase: effect on kitten growth rate. *Am. J. Clin. Nutr.* **49:** 457–463.
74. Howles, P., B. Wagner, and L. Davis. 1998. Bile salt stimulated lipase is required for proper digestion and absorption of milk triglycerides in neonatal mice. (Abstract). *FASEB J.* **12:** A851.
75. Howles, P. N., G. N. Stemmerman, C. M. Fenoglio-Preiser, and D. Y. Hui. 1999. Carboxyl ester lipase activity in milk prevents fat-derived intestinal injury in neonatal mice. *Am. J. Physiol.* **277:** G653–G661.
76. Crissinger, K. D., D. L. Burney, O. R. Velasquez, and E. Gonzalez. 1994. An animal model of necrotizing enterocolitis induced by infant formula and ischemia in developing piglets. *Gastroenterol.* **106:** 1215-1222.
77. Velasquez, O. R., P. Tso, and K. D. Crinninger. 1993. Fatty acid-induced injury in developing piglet intestine: Effect of degree of saturation and carbon chain length. *Pediatr. Res.* **33:** 543–547.
78. Velasquez, O. R., K. Henninger, M. Fowler, P. Tso, and K. D. Crissinger. 1993. Oleic acid-induced mucosal injury in developing piglet intestine. *Am. J. Physiol.* **264:** G576–G582.
79. Velasquez, O. R., A. R. Place, P. Tso, and K. D. Crissinger. 1994. Developing intestine is injured during absorption of oleic acid but not its ethyl ester. *J. Clin. Invest.* **93:** 479–485.
80. Udall, J. N., P. Colony, L. Fritze, K. Pang, J. S. Trier, and W. A. Walker. 1981. Development of gastrointestinal mucosal barrier. II. The effect of natural versus artificial feeding on intestinal permeability to macromolecules. *Pediatr. Res.* **15:** 245–249.
81. Weaver, L. T., L. Landymore-Lim, and A. Lucas. 1991. Neonatal gastrointestinal growth and function: are they regulated by composition of feeds. *Biol. Neonate.* **59:** 336–345.
82. Musemeche, C. A., R. P. Pizzini, and R. J. Andrassy. 1993. Intestinal ischemia in the newborn: the role of intestinal maturation. *J. Surg. Res.* **55:** 595–598.
83. Rowe, M. I., K. K. Reblock, A. G. Kurkchubasche, and P. J. Healey. 1994. Necrotizing enterocolitis in the extremely low birth weight infant. *J. Pediatr. Surg.* **29:** 987–991.
84. Carey, M. C., D. M. Small, and C. M. Bliss. 1983. Lipid digestion and absorption. *Annu. Rev. Physiol.* **45:** 651–677.
85. Chen, Q., B. Sternby, and A. Nilsson. 1989. Hydrolysis of triacylglycerol arachidonic and linoleic acid ester bonds by human pancreatic lipase and carboxyl ester lipase. *Biochim. Biophys. Acta.* **1004:** 372–385.
86. Chen, Q., B. Sternby, B. Akesson, and A. Nilsson. 1990. Effects of human pancreatic lipase-colipase and carboxyl ester lipase on eicosapentaenoic and arachidonic acid ester bonds of triacylglycerols rich in fish oil fatty acids. *Biochim. Biophys. Acta.* **1044:** 111–117.
87. Chen, Q., L. Blackberg, A. Nilsson, B. Sternby, and O. Hernell. 1994. Digestion of triacylglycerols containing long-chain polyenoic fatty acids in vitro by colipase-dependent pancreatic lipase and human milk bile salt-stimulated lipase. *Biochim. Biophys. Acta.* **1210:** 239–243.
88. Hernell, O., L. Blackberg, Q. Chen, B. Sternby, and A. Nilsson. 1993. Does the bile salt-stimulated lipase of human milk have a role in the use of the milk long-chain polyunsaturated fatty acids. *J. Pediatr. Gastroenterol. Nutr.* **16:** 426–431.
89. Mattson, F. H., and R. A. Volpenhein. 1972. Rate and extent of absorption of the fatty acids of fully esterified glycerol, erythritol, and sucrose as measured in thoracic duct cannulated rats. *J. Nutr.* **102:** 1177–1180.
90. Howles, P. N., C. P. Carter, and D. Y. Hui. 1996. Dietary free and esterified cholesterol absorption in cholesterol esterase (bile salt-stimulated lipase) gene-targeted mice. *J. Biol. Chem.* **271:** 7196–7202.
91. Weng, W., L. Li, A. M. van Bennekum, S. H. Potter, E. H. Harrison, W. S. Blaner, J. L. Breslow, and E. A. Fisher. 1999. Intestinal absorption of dietary cholesteryl ester is decreased but retinyl ester absorption is normal in carboxyl ester lipase knockout mice. *Biochemistry.* **38:** 4143–4149.
92. Gallo, L. L., S. B. Clark, S. Myers, and G. V. Vahouny. 1984. Cholesterol absorption in rat intestine: Role of cholesterol esterase and acyl coenzyme A:cholesterol acyltransferase. *J. Lipid Res.* **25:** 604–612.
93. McKean, M. L., T. J. Commons, M. S. Berens, P. L. Hsu, D. M. Ackerman, K. E. Steiner, and S. J. Adelman. 1992. Effects of inhibitors of pancreatic cholesterol ester hydrolase (PCEH) on 14-C cholesterol absorption in animals models. *FASEB J.* **6:** A1388 (Abstract).
94. Krause, B. R., D. R. Sliskovic, M. Anderson, and R. Homan. 1998. Lipid-lowering effects of WAY-121,898, an inhibitor of pancreatic cholesteryl ester hydrolase. *Lipids.* **33:** 489–498.
95. Watt, S. M., and W. J. Simmonds. 1981. The effect of pancreatic diversion on lymphatic absorption and esterification of cholesterol in the rat. *J. Lipid Res.* **22:** 157–165.
96. Myers-Payne, S. C., D. Y. Hui, H. L. Brockman, and F. Schroeder. 1995. Cholesterol esterase: A cholesterol transfer protein. *Biochemistry.* **34:** 3942–3947.
97. Ikeda, I., R. Matsuoka, T. Hamada, K. Mitsui, S. Imabayashi, A. Uchino, M. Sato, E. Kuwano, T. Itamura, K. Yamada, K. Tanaka, and K. Imaizumi. 2002. Cholesterol esterase accelerates intestinal cholesterol absorption. *Biochim. Biophys. Acta.* **1571:** 34–44.
98. Young, S. C., and D. Y. Hui. 1999. Pancreatic lipase-colipase mediated triglyceride hydrolysis is required for cholesterol transport from lipid emulsions to intestinal cells. *Biochem. J.* **339:** 615–620.
99. Richmond, B. L., A. C. Boileau, S. Zheng, K. W. Huggins, N. A. Granholm, P. Tso, and D. Y. Hui. 2001. Compensatory phospholipid digestion is required for cholesterol absorption in pancreatic phospholipid A(2) deficient mice. *Gastroenterology.* **120:** 1193–1202.
100. Pind, S., and A. Kuksis. 1991. Further characterization of a novel phospholipase B (Phospholipase A2-lysophospholipase) from intestinal brush border membranes. *Biochem. Cell Biol.* **69:** 346–357.
101. Takemori, H., F. N. Zolotaryov, L. Ting, T. Urbain, T. Komatsubara, O. Hatano, M. Okamoto, and H. Tojo. 1998. Identification of functional domains of rat intestinal phospholipase B/lipase. Its cDNA cloning, expression, and tissue distribution. *J. Biol. Chem.* **273:** 2222–2231.
102. Tojo, H., T. Ichida, and M. Okamoto. 1998. Purification and characterization of a catalytic domain of rat intestinal phospholipase B/lipase associated with brush border membranes. *J. Biol. Chem.* **273:** 2214–2221.
103. Sawai, T., R. A. Drongowski, R. W. Lampman, A. G. Coran, and C. M. Harmon. 2001. The effect of phospholipids and fatty acids on tight junction permeability and bacterial translocatioin. *Pediatr. Surg. Int.* **17:** 269–274.
104. Howles, P., B. Wagner, B. Lewandowski, S. Zheng, A. Gupta, C. Carter, D. Y. Hui, and P. Tso. 2000. Pancreatic carboxyl ester lipase is important for vitamin A absorption (Abstract). *Gastroenterology.* **118:** A196.
105. Fernandez, E., and B. Borgstrom. 1990. Intestinal absorption of retinol and retinyl palmitate in the rat. *Lipids.* **25:** 549–552.
106. van Bennekum, A. M., E. A. Fisher, W. S. Blaner, and E. H. Harrison. 2000. Hydrolysis of retinyl esters by pancreatic triglyceride lipase. *Biochemistry.* **39:** 4900–4906.
107. Field, F. J., H. Chen, E. Born, B. Dixon, and S. Mathur. 1993. Release of ceramide after membrane sphingomyelin hydrolysis decreases the basolateral secretion of triacylglycerol and apolipoprotein B in cultured human intestinal cells. *J. Clin. Invest.* **92:** 2609–2619.
108. Chen, H., E. Born, S. N. Mathur, F. C. Johlin, and F. J. Field. 1992. Sphingomyelin content of intestinal cell membranes regulates cholesterol absorption. Evidence for pancreatic and intestinal cell sphingomyelinase activity. *Biochem. J.* **286:** 771–777.
109. Field, F. J., E. Born, and S. N. Mathur. 1995. Triacylglycerol-rich lipoprotein cholesterol is derived from the plasma membrane in Caco-2 cells. *J. Lipid Res.* **36:** 2651–2660.
110. Field, F. J., E. Born, H. Chen, S. Murthy, and S. N. Mathur. 1995. Esterification of plasma membrane cholesterol and triacylglycerol-rich lipoprotein secretion in Caco-2 cells: possible role of p-glycoproteins. *J. Lipid Res.* **36:** 1533–1543.
111. Field, F. J., E. Born, and S. N. Mathur. 1997. Effect of micellar β-sitosterol on cholesterol metabolism in Caco-2 cells. *J. Lipid Res.* **38:** 348–360.
112. Slotte, J. P., and E. L. Bierman. 1988. Depletion of plasma membrane sphingomyelin rapidly alters the distribution of cholesterol between plasma membranes and intracellular cholesterol pools in cultured fibroblasts. *Biochem. J.* **250:** 653–658.
113. Gupta, A. K., and H. Rudney. 1991. Plasma membrane sphingomyelin and the regulation of HMG-CoA reductase activity and cholesterol biosynthesis in cell cultures. *J. Lipid Res.* **32:** 125–136.

114. Fukunaga, T., M. Nagahama, K. Hatsuzawa, K. Tani, A. Yamamoto, and M. Tagaya. 2000. Implication of sphingolipid metabolism in the stability of the Golgi apparatus. *J. Cell Sci.* **113:** 3299–3307.
115. Rosenwald, A. G., and R. E. Pagano. 1993. Inhibition of glycoprotein traffic through the secretory pathway by ceramide. *J. Biol. Chem.* **268:** 4577–4579.
116. Rea, T. J., R. B. DeMattos, and M. E. Pape. 1993. Hepatic expression of genes regulating lipid metabolism in rabbits. *J. Lipid Res.* **34:** 1901–1910.
117. Brodt-Eppley, J., P. White, S. Jenkins, and D. Y. Hui. 1995. Plasma cholesterol esterase level is a determinant for an atherogenic lipoprotein profile in normolipidemic human subjects. *Biochim. Biophys. Acta.* **1272:** 69–72.
118. Harrison, E. H. 1988. Bile salt-dependent, neutral cholesteryl ester hydrolase of rat liver: Possible relationship with pancreatic cholesteryl ester hydrolase. *Biochim. Biophys. Acta.* **963:** 28–34.
119. Shamir, R., E. J. Johnson, K. Morlock-Fitzpatrick, R. Zolfaghari, L. Li, E. Mas, D. Lambardo, D. W. Morel, and E. A. Fisher. 1996. Pancreatic carboxyl ester lipase: A circulating enzyme that modifies normal and oxidized lipoproteins in vitro. *J. Clin. Invest.* **97:** 1696–1704.
120. Campbell, C. B., C. McGuffie, and L. W. Powell. 1975. The measurement of sulphated and non-sulphated bile acids in serum using gas liquid chromatography. *Clin. Chim. Acta.* **63:** 249–262.
121. Costarelli, V., and T. A. B. Sanders. 2001. Acute effects of dietary fat composition on postprandial plasma bile acid and cholecystokinin concentrations in healthy premenopausal women. *Br. J. Nutr.* **86:** 471–477.
122. Angelin, B., I. Bjorkhem, K. Einarsson, and S. Ewerth. 1982. Hepatic uptake of bile acids in man. *J. Clin. Invest.* **70:** 724–731.
123. Natarajan, R., S. Ghosh, S., and W. M. Grogan. 1996. Catalytic properties of the purified rat hepatic cytosolic cholesteryl ester hydrolase. *Biochem. Biophys. Res. Commun.* **225:** 413-419.
124. Natarajan, R., S. Ghosh, and W. M. Grogan. 1997. Age-related changes in catalytic activity, enzyme mass, mRNA, and subcellular distribution of hepatic neutral cholesterol ester hydrolase in female rats. *Lipids.* **32:** 463–470.
125. Natarajan, R., S. Ghosh, and W. M. Grogan. 1998. Molecular cloning of the promoter for rat hepatic neutral cholesterol ester hydrolase: evidence for transcriptional regulation by sterols. *Biochem. Biophys. Res. Commun.* **243:** 349–355.
126. Ghosh, S., R. Natarajan, W. M. Pandak, P. B. Hylemon, and W. M. Grogan. 1998. Regulation of hepatic neutral cholesteryl ester hydrolase by hormones and changes in cholesterol flux. *Am. J. Physiol.* **274:** G662–G668.
127. Hornick, C. A., D. Y. Hui, and J. G. DeLamatre. 1997. A role for retrosomes in intracellular cholesterol transport from endosomes to the plasma membrane. *Am. J. Physiol.* **273:** C1075–C1081.
128. Winkler, K. E., E. H. Harrison, J. B. Marsh, J. M. Glick, and A. C. Ross. 1992. Characterization of a bile salt-dependent cholesteryl ester hydrolase activity secreted from HepG2 cells. *Biochim. Biophys. Acta.* **1126:** 151–158.
129. Li, F., Y. Huang, and D. Y. Hui. 1996. Bile salt stimulated cholesterol esterase increases uptake of high density lipoprotein-associated cholesteryl esters by HepG2 cells. *Biochemistry.* **35:** 6657–6663.
130. Camarota, L. M., J. C. Chapman, D. Y. Hui, and P. N. Howles. 2002. Carboxyl ester lipase effects selective uptake and hydrolysis of HDL-cholesterol esters (Abstract). *Gastroenterology.* **122 (suppl. 1):** A625.
131. Kothari, H. V., B. F. Miller, and D. Kritchevsky. 1973. Aortic cholesterl esterase: characteristics of normal rat and rabbit enzyme. *Biochim. Biophys. Acta.* **296:** 446–454.
132. Kothari, H. V., and D. Kritchevsky. 1975. Purification and properties of aortic cholesteryl ester hydrolase. *Lipids.* **10:** 322–330.
133. Witztum, J. L., and D. Steinberg. 1991. Role of oxidized low density lipoprotein in atherogenesis. *J. Clin. Invest.* **88:** 1785–1792.
134. Witztum, J. L. 1994. The oxidation hypothesis of atherosclerosis. *Lancet.* **344:** 793–795.
135. Portman, O. W., and M. Alexander. 1969. Lysophosphatidylcholine concentrations and metabolism in aortic intima plus inner media: effect of nutritionally induced atherosclerosis. *J. Lipid Res.* **10:** 158–165.
136. Kugiyama, K., S. A. Kerns, J. D. Morrisett, R. Roberts, and P. D. Henry. 1990. Impairment of endothelium-dependent arterial relaxation by lysolecithin in modified low density lipoproteins. *Nature.* **344:** 160–162.
137. Quinn, M. T., S. Parthasarathy, and D. Steinberg. 1988. Lysophosphatidylcholine: A chemotactic factor for human monocytes and its potential role in atherogenesis. *Proc. Natl. Acad. Sci. USA.* **85:** 2805–2809.
138. Kume, N., M. I. Cybulsky, and M. A. Gimbrone. 1992. Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. *J. Clin. Invest.* **90:** 1138–1144.
139. Sakai, M., A. Miyazaki, H. Hakamata, T. Sasaki, S. Yui, M. Yamazaki, M. Shichiri, and S. Horiuchi. 1994. Lysophosphatidylcholine plays an essential role in the mitogen effect of oxidized low density lipoprotein on murine macrophages. *J. Biol. Chem.* **269:** 31430–31435.
140. Parthasarathy, S., and J. Barnett. 1990. Phospholipase A-2 activity of low density lipoprotein: Evidence for an intrinsic phospholipase A-2 activity of apoprotein B-100. *Proc. Natl. Acad. Sci. USA.* **87:** 9741–9745.
141. Elinder, L. S., A. Dumitrescu, P. Larsson, U. Hedin, J. Frostegard, and H. E. Claesson. 1997. Expression of phospholipase A2 isoforms in human normal and atherosclerotic arterial wall. *Arterioscler. Thromb. Vasc. Biol.* **17:** 2257–2263.
142. Hurt-Camejo, E., S. Andersen, R. Standal, B. Rosengren, P. Sartipy, E. Stadberg, and B. Johansen. 1997. Localization of nonpancreatic secretory phospholipase A2 in normal and atherosclerotic arteries. Activity of the isolated enzyme on low density lipoproteins. *Arterioscler. Thromb. Vasc. Biol.* **17:** 300–309.
143. Romano, M., E. Romano, S. Bjorkerud, and E. Hurt-Camejo. 1998. Ultrastructural localization of secretory type II phospholipase A2 in atherosclerotic and nonatherosclerotic regions of human arteries. *Arterioscler. Thromb. Vasc. Biol.* **18:** 519–525.
144. Ivandic, B., F. C. deBeer, M. C. deBeer, L. Castellani, N. Leitinger, S. Y. Hama, J. Lee, X. P. Wang, M. Navab, A. M. Fogelman, and A. J. Lusis. 1996. Transgenic mice overexpressing secretory phospholipase A2 develop markedly increased aortic fatty streak lesions. *Circulation.* **94:** I-152
145. Ivandic, B., L. W. Castellani, X. P. Wang, J. H. Qiao, M. Mehrabian, M. Navab, A. M. Fogelman, D. S. Grass, M. E. Swanson, M. C. de Beer, F. de Beer, and A. J. Lusis. 1999. Role of Group II secretoary phospholipase A2 in atherosclerosis. 1. Increased atherogenesis and altered lipoproteins in transgenic mice expressing group IIa phospholipase A2. *Arterioscler. Thromb. Vasc. Biol.* **19:** 1284–1290.
146. Schissel, S. L., J. Tweedie-Hardman, J. H. Rapp, G. Graham, K. J. Williams, and I. Tabas. 1996. Rabbit aorta and human atherosclerotic lesions hydrolyze the sphingomyelin of retained low density lipoprotein. *J. Clin. Invest.* **98:** 1455–1464.
147. Auge, N., I. Escargueil-Blanc, I. Lajoie-Mazenc, I. Suc, N. Andrieu-Abadie, M. T. Pieraggi, M. Chatelut, J. C. Thiers, J. P. Jaffrezou, G. Laurent, T. Levade, A. Negre-Salvayre, and R. Salvayre. 1998. Potential role for ceramide in mitogen activated protein kinase activation and proliferation of vascular smooth muscle cells induced by oxidized low density lipoprotein. *J. Biol. Chem.* **273:** 12893–12900.
148. Auge, N., M. Nikolova-Karakashian, S. Carpentier, S. Parthasarathy, A. Negre-Salvayre, R. Salvayre, A. H. Merrill, and T. Levade. 1999. Role of sphingosine 1-phosphate in the mitogenesis induced by oxidized low density lipoprotein in smooth muscle cells via activation of sphingomyelinase, ceramidase, and sphingosine kinase. *J. Biol. Chem.* **274:** 21533–21538.
149. Bhunia, A. K., H. Han, A. Snowden, and S. Chatterjee. 1997. Redox-regulated signaling by lactosylceramide in the proliferation of human aortic smooth muscle cells. *J. Biol. Chem.* **272:** 15642–15649.
150. Chatterjee, S. 1998. Sphingolipids in atherosclerosis and vascular biology. *Arterioscler. Thromb. Vasc. Biol.* **18:** 1523–1533.