Absorption and lipoprotein transport of sphingomyelin

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Abstract **Dietary sphingomyelin (SM) is hydrolyzed by intestinal alkaline sphingomyelinase and neutral ceramidase to sphingosine, which is absorbed and converted to palmitic acid and acylated into chylomicron triglycerides (TGs). SM digestion is slow and is affected by luminal factors such as bile salt, cholesterol, and other lipids. In the gut, SM and its metabolites may influence TG hydrolysis, cholesterol absorption, lipoprotein formation, and mucosal growth. SM accounts for ~20% of the phospholipids in human plasma lipoproteins, of which two-thirds are in LDL and VLDL. It is secreted in chylomicrons and VLDL and transferred into HDL via the ABCA1 transporter. Plasma SM increases after periods of large lipid loads, during suckling, and in type II hypercholesterolemia, cholesterol-fed animals, and apolipoprotein E-deficient mice. SM is thus an important amphiphilic component when plasma lipoprotein pools expand in response to large lipid loads or metabolic abnormalities. It inhibits lipoprotein lipase and LCAT as well as the interaction of lipoproteins with receptors and counteracts LDL oxidation. The turnover of plasma SM is greater than can be accounted for by the turnover of LDL and HDL particles. Some SM must be degraded via receptor-mediated catabolism of chylomicron and VLDL remnants and by scavenger receptor class B type I receptor-mediated transfer into cells.—**Nilsson, Å., and R-D. Duan. **Absorption and lipoprotein transport of sphingomyelin. *J. Lipid Res.* 2006.** 47: **154–171.**

Supplementary key words chylomicron • very low density lipoprotein • low density lipoprotein • sphingomyelinase • ceramidase • phospholipid transfer protein

Sphingomyelin (SM) in mammalian cells is colocalized with cholesterol mainly in the plasma membrane and in lysosomal and Golgi membranes. It interacts strongly with cholesterol, and the regulation of SM and cholesterol metabolism are in part coordinated (1, 2). In plasma lipoproteins, SM is the second most abundant polar lipid after phosphatidylcholine (PC). The size of the plasma lipoprotein SM pool in humans is 1–1.5 g, of which approximately two-thirds are in apolipoprotein B (apoB)-containing triglyceride (TG)-rich lipoproteins and LDL. The SM content in most extraneural tissues is 1–2 g/kg. Factors regulating plasma SM concentration have received little attention. It was early shown that the level of SM is increased in hypercholesterolemia and that SM-rich lipoproteins accumulate in arteriosclerotic lesions (3–5). Plasma SM is thus a risk factor for ischemic heart disease (6), and the apoE-deficient (apoE$^{-/-}$) mouse, which accumulates SM-rich remnant particles in blood (7), has emerged as an important model for studying the role of SM in atherogenesis. The effects of lipoprotein SM in the arterial wall during atherogenesis may be related both to the modification of lipoproteins and to the generation of sphingolipid messengers (i.e., ceramide, ceramide-1-phosphate, sphingosine, and sphingosine-1-phosphate) initiated by SM hydrolysis (8–10). The role of SM metabolites in cell signaling has been the subject of several recent reviews (11–16). Sphingolipid signals are triggered by numerous stimuli and mediate effects on cell growth and apoptosis and on the activities of inflammatory cells that may be pathogenic as well as protective during the development of the arteriosclerotic lesion (17). Recently, myriocin, an inhibitor of SM biosynthesis, was found to selectively decrease plasma SM and to decrease arteriosclerosis in apoE$^{-/-}$ mice (18, 19), and this finding has been the subject of an editorial comment (20).

This review focuses on the absorption and transport of SM and on the biological effects of SM and its metabolites that may be exerted during these processes.

ABSORPTION OF SM

Pathways and course of digestion and absorption

Humans on an ordinary Western diet ingest 0.3–0.4 g of sphingolipids per day, of which SM in meat, milk, egg products, and fish (21–23) is a large part. The suckling baby ingesting milk consumes only ~150 mg of SM per day. SM accounts for ~2% of the phospholipids in human bile, which means that 100–200 mg is delivered to the gut

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Abbreviations: apoB, apolipoprotein B; BSSL, bile salt-stimulated lipase; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; ER, endoplasmic reticulum; IDL, intermediate density lipoprotein; LRP, low density lipoprotein receptor-related protein; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PLTP, phospholipid transfer protein; SM, sphingomyelin; SMase, sphingomyelinase; SR-BI, scavenger receptor class B type I; TG, triglyceride.

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every day (24), mainly the palmitoyl and stearoyl species (25). The brush border of the mucosal cells is rich in sphingolipids and is an additional source of endogenous SM. In dog and human chyle chylomicrons, SM accounts for ~5% and 7%, respectively, of the total polar lipids (26, 27). Therefore, the question was early raised whether dietary SM could be absorbed intact and contribute to the chylomicron and plasma SM pools. Studies on lymphatic duct-cannulated rats that were fed [^{3}H]sphingosine- or [^{14}C]stearic acid-labeled SM, however, showed that little or no labeled SM was absorbed intact into the chyle (28). SM was sequentially hydrolyzed to ceramide and then to sphingosine and free fatty acids (29). Similarly, after feeding [^{3}H]palmitoyl-sphingosine, no evidence was obtained for the incorporation of intact dietary ceramide into chyle lipoproteins either as ceramide or as SM, but the [^{3}H]palmitic acid appears primarily in chyle TG. A small amount of radioactive ceramide was found in intestinal tissue, but it was not confirmed whether it was located intracellularly as a result of a slow absorption of intact long-chain ceramide or just associated to the mucosal surface. In contrast to SM and ceramide, free sphingosine is well absorbed and rapidly metabolized in the mucosal cells. Most of the absorbed sphingosine is converted to palmitic acid and incorporated into chylomicrons. The key enzymes in this reaction [i.e., sphingosine kinase (30), sphingosine-1-phosphate lyase, and the palmitaldehyde oxidase] are all found at high levels in the intestinal mucosa (31). A smaller portion of the sphingoid bases are reincorporated into mucosal ceramide and more complex sphingolipids (28, 32), and some of this newly formed ceramide appears as chyle ceramide rather than chyle SM (28). The pathways for the metabolism of dietary SM in the gut are summarized in **Fig. 1**.

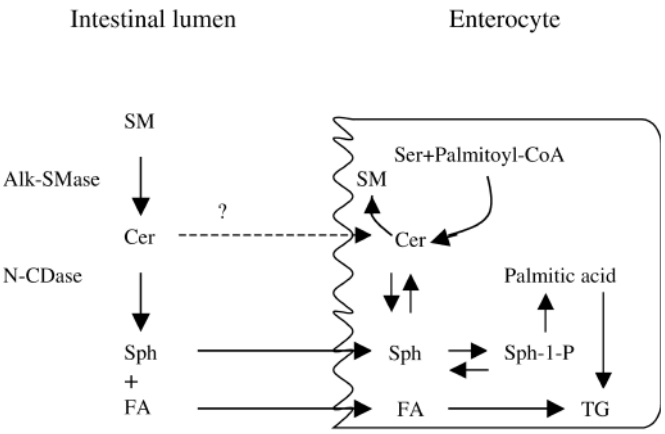

Fig. 1. Pathways for the digestion and absorption of sphingomyelin (SM). Alkaline sphingomyelinase (Alk-SMase) and neutral ceramidase (N-CDase) sequentially hydrolyze SM to ceramide (Cer), sphingosine (Sph), and free fatty acids (FA). Sphingosine is well absorbed and converted to chylomicron palmitic acid. A small part is incorporated into mucosa ceramide and more complex sphingolipids. The endogenous synthesis of SM, starting from serine (Ser) and palmitoyl-CoA, is also indicated.

Intestinal alkaline sphingomyelinase and neutral ceramidase

The initial step in the digestion of dietary SM in the gut is catalyzed by intestinal alkaline sphingomyelinase (SMase), which is present in all species examined except guinea pig (33) and is also found in human bile (34). Its longitudinal distribution shows a maximum in the jejunum. Recently, the enzyme was purified (35, 36) and cloned from both human and rat and found to lack homology to known neutral and acid SMases but to be related to the nucleotide-phosphodiesterase family (37, 38). However, it does not hydrolyze nucleotides like other nucleotide-phosphodiesterases but only choline phospholipids, preferring SM. The enzyme is strictly bile salt-dependent, taurocholate and taurochenodeoxycholate being the most effective stimulators. It is resistant to pancreatic proteases and can be released from the mucosa by bile salts and by tryptic digestion of a C-terminal peptide that anchors the enzyme to the brush border (39). Ceramide formed by alkaline SMase is further hydrolyzed by intestinal neutral ceramidase, which was recently purified from rat intestinal mucosa and characterized (40). The enzyme is identical to or highly homologous to the neutral ceramidase identified previously in the apical membrane of renal tubular cells (41) and the one shown by Choi et al. (42) to be expressed at a high level in the gut and located at the brush border. Although the bile salt-stimulated lipase (BSSL) present in human milk and in pancreatic juice was shown to hydrolyze ceramide (43), the mucosal ceramidase is probably more important for the digestion of ceramide formed from dietary SM than is BSSL (44). The neutral ceramidase has a longitudinal distribution that coincides with the main site of ceramide digestion (i.e., the middle and lower small intestine), where the ceramidase activity is not decreased in BSSL$^{-/-}$ mice (45). The level of BSSL is highest in the upper part of the intestine, where the action of BSSL on ceramide is expected to be inhibited by the presence of glycerolipids (43).

Factors affecting SM digestion

The course of SM digestion and thus the exposure of distal small intestine and colon to SM and its metabolites can be influenced by multiple factors, such as the amount of SM given, the presence of bile salts and other lipids, and the levels of the enzymes involved in SM digestion. Early studies indicated an extended course and a limited capacity of SM digestion. The recovery of SM [^{14}C]stearic acid in chyle was lower than is generally obtained for glycerolipid fatty acids, and ~25% of the sphingosine part appeared in feces, mainly as ceramide (28). This proportion, however, did not increase when the given dose was increased from 1 to 25 mg. Nyberg et al. (46) fed 0.2 to 32 μmol of milk SM together with [^{3}H]sphinganine-labeled dihydrosphingomyelin and analyzed the tissue and the content of four different levels of small intestine after 1–8 h as well as feces collected during 24 h. Increasing the dose increased the proportion and amount of undigested SM in the lower half of the gut, decreased the reincorpo-

ration of fatty acids in gut and liver tissue, and increased the output of SM and ceramide in feces.

In a human study, Hertervig (47) found that feeding a dose of 250 mg of milk SM in a standardized meal significantly increased the output of both ceramide and intact SM in ileostomy content in humans, indicating an incomplete digestion and absorption of SM. Although the factors that are rate-limiting for the digestion of SM and ceramide are not fully understood, some relevant observations have been made. When radiolabeled SM was sonicated together with cholesterol or sitosterol and given orally to rats, the digestion of SM was delayed and colonic exposure was increased (48). Studies in vitro using purified alkaline SMase showed that the presence of either polar or nonpolar glycerolipids, or sterols, but not of free fatty acids, inhibited SM hydrolysis by purified alkaline SMase (49). The higher alkaline SMase in jejunum and ileum than in duodenum as well as the presence of higher concentrations of dietary glycerolipids in the upper part may thus favor the location of SM digestion primarily to the middle and lower small intestine.

The regulation of alkaline SMase and ceramidase expression has not been studied extensively. Data from some studies in our laboratory indicate that expression of alkaline SMase and ceramidase can be affected by dietary factors and drugs. It was found that alkaline SMase was increased by psyllium and ursodeoxycholic acid (50, 51) and decreased by a fat-rich diet. Psyllium also decreased ceramidase activity and may thus increase ceramide levels in the gut. Variations in the key apoptotic enzyme caspase 3 correlated positively to alkaline SMase but if anything negatively to acid SMase (52). Insoluble fiber increased acid but not alkaline SMase.

SM digestion in the neonate

Because SM accounts for almost 40% of the polar lipids in human and cow milk, it is important to know whether the neonate can utilize the different components of the SM molecule and whether metabolites formed during the digestion of milk SM may influence normal gut function, maturation, and differentiation. This question has not been thoroughly investigated. Both alkaline SMase and neutral ceramidase, however, were early found to be present in human meconium (29), and studies on fetal and neonatal rats showed that alkaline SMase was promptly expressed soon before birth and the levels kept increasing to a plateau at 4 weeks after birth (53). Three week old suckling pigs had high levels of alkaline SMase in jejunum and ileum (54). The enzymes that digest SM are thus expressed when suckling starts. Acid SMase was shown to be secreted in milk (43). Gastric and duodenal intubation studies of 11 suckling newborns, however, indicated that digestion of milk SM in stomach and upper duodenum is negligible, whereas three jejunal samples and two ileal samples obtained from babies undergoing surgery indicated ceramide formation in these regions of the gut (54). The transfer of nervonic acid, which occurs specifically in sphingolipids, to tissues of newborn rats from mother's milk supports the notion that milk SM is indeed digested and the fatty acids absorbed (55).

During the period from birth to weanling, there is a continuous change in the lipid composition of both the brush border and the basolateral membrane of the gut epithelium. Whereas the brush border increases its cholesterol and SM content during suckling, there is a progressive decrease in the SM/PC ratio in the basolateral membrane (56, 57). The physiological relevance of these changes and whether the supply of SM in milk contributes to these changes are unknown.

EFFECTS OF DIETARY SPHINGOLIPIDS IN THE INTESTINE

Effects on lipid and cholesterol absorption

Because of the extended and slow digestion, SM may influence the course of the digestion and absorption of other lipids by physical effects in the gut lumen or via biological effects of the metabolites. It is known that the rate of hydrolysis of emulsified TG by pancreatic colipase-dependent lipase may be inhibited by the presence of phospholipids on the polar surface. A previous study by Borgström (58) showed that simultaneous treatment of TG emulsion by phospholipase A_2 significantly shortened the lag time of TG hydrolysis by pancreatic lipase. Patton and Carey (59) found that bile salt phospholipid micelles, including SM-bile salt micelles, could displace colipase from gum arabic emulsified substrate and inhibit the lipolysis of TG. Therefore, it is possible that the presence of SM and the slow hydrolysis of SM extend the course of TG hydrolysis, which has been demonstrated in vitro.

SM has been shown to inhibit cholesterol absorption both in vivo and in CaCo2 cell cultures. When equimolar amounts of cholesterol and SM were fed orally to rats as sonicated dispersions, only 10% of the radioactive cholesterol was absorbed, as estimated by the dual isotope method (48). The inhibitory effect was mutual in the sense that cholesterol and plant sterols also decreased the digestion and absorption of SM (48). Incorporation of cholesterol into SM-PC vesicles was shown to have profound effects on detergent-induced phase transitions, indicating a physicochemical cause of the absorption inhibition (60). In support of this possibility, Eckhardt et al. (61) correlated the effect of SM on the partitioning of cholesterol from bile salt solutions to polymerized silicone to an inhibitory effect of SM on cholesterol uptake in vivo and in differentiated CaCo2 cells. When cholesterol absorption was studied in lymphatic duct-cannulated rats, both egg SM and milk SM decreased cholesterol absorption, milk SM being most effective (62, 63). Interestingly, the lymphatic output of SM decreased rather than increased during SM feeding. Whether this reflects a decreased incorporation of SM into chylomicrons secondary to the decreased cholesterol absorption is unknown. Studies by Kirby et al. (45) and by Field and colleagues (64, 65) showed an increased uptake of cholesterol by CaCo2 cells after hydrolysis of SM or ceramide. Recently, sphingosine

was shown to decrease cholesterol absorption in CaCo2 cells and to downregulate the Niemann-Pick-Like Protein 1, which has been implicated as a key protein in cholesterol absorption and a target for the cholesterol absorption inhibitor ezetimibe (66). Not only the physical interaction of SM in gut lumen but also regulatory influences of the metabolites may thus influence cholesterol absorption and lipoprotein composition.

The conclusion is that dietary SM may influence cholesterol absorption and lipoprotein metabolism in the gut by several mechanisms. The important questions of whether SM may influence the absorption of endogenous cholesterol and postprandial lipoprotein metabolism in humans have not been answered.

Effects on cell growth and differentiation

Because ceramide and sphingosine are well-known regulators of cell growth, differentiation, and apoptosis, the questions have been asked whether the metabolites formed from dietary SM may influence the cell cycle of the gut epithelium under normal and tumorigenic conditions, and whether sphingolipid metabolites regulate normal proliferation and differentiation in the crypt cell progenitor compartment and cell fate along the crypt villous axis. Choi et al. (42) found increased apoptosis in absorptive villous cells after feeding a short-chain ceramide, if ceramidase was blocked, and suggested that the brush border ceramidase is needed to protect absorptive cells from the apoptotic effect of absorbed ceramide. Whether this can be applied to long-chain ceramides that are expected to be less effectively absorbed than the short-chain ceramide is not known.

SM in the milk may have important roles in the mucosal development of intestine in the newborn. Milk replacement formulas regularly contain far less SM than does mother's milk. Motouri et al. (67) fed SM-enriched milk formulas or an equivalent amount of PC to suckling rat pups and found lower lactase levels, a larger Auerbach nerve plexus, and restriction of vacuolated epithelial cells to the villous tip in the SM group. More extensive studies in this area, including examination of the intestinal immune system, are needed.

As reviewed by Schmelz (68) and by Duan (69), several pieces of evidence indicate that sphingolipid metabolism may mediate anticarcinogenic mechanisms in the colon. Feeding sphingolipids to animals treated with the chemical carcinogen dimethylhydrazine or to MIN mice, an animal model for human familial adenomatous polyposis, showed an inhibitory effect on both the initiation and propagation of colon tumors. Studies on colon cancer HT9 cells showed that sphingosine induced apoptosis and the inhibition of proliferation by interacting with the β-catenin system (70). Antitumor effects of SM in the gut may thus be exerted by increasing the exposure of the gut to its degradation products ceramide and/or sphingosine. Although alkaline SMase is expressed at the highest level in the middle and lower small intestine (71), it is found also in the colon mucosa. The enzyme activity was low in many colon tumors, in mucosa from familial adenomatous polyposis patients (72, 73), and in stools of colorectal cancer patients (74). Purified alkaline SMase was shown to inhibit the proliferation of colon cancer HT29 cells (75). Recently, we found that this cell line has an inactivating mutation in the alkaline SMase gene (76). Under normal conditions, notably substantial amounts of alkaline SMase and intestinal ceramidase reach the colon in the intestinal content. These enzymes may protect the colonic mucosa from tumorigenesis by generating ceramide and sphingosine in the colon.

SM is present in bile, where it accounts for a small minority of the total phospholipids in most species examined (24). There is convincing evidence that phospholipids in bile prevent cytotoxicity induced by bile salts (77). Although SM accounts for a small part of the bile phospholipids, it may contribute to this effect (78), particularly in the lower small intestine, where some undigested SM persists but where most other lipids but not the bile salts have been absorbed. SM was also found to prevent deoxycholate-induced apoptosis and hyperproliferation in CaCo2 cells. This provides an alternative mechanism by which SM may have anticarcinogenic effects in colon (79).

Conclusion

SM digestion is an extended process with limited capacity and is catalyzed primarily by alkaline SMase and neutral ceramidase, which are in part released into and act in the gut lumen. The generated sphingosine is well absorbed and effectively metabolized in the mucosal cells. SM and its metabolites in the gut may inhibit TG hydrolysis and cholesterol absorption and affect cell proliferation and maturation. The slow and extended digestion of SM makes it possible to increase the exposure of the whole gut to SM and its metabolites by dietary means. However, many questions regarding the biological effects or the mechanism of the effects remain to be solved, such as the factors limiting ceramide assimilation and the effects on cell growth and differentiation in both the neonate and the adult. It is still not known whether SM has a significant effect on postprandial lipoproteins in humans.

SM IN PLASMA LIPOPROTEINS

SM levels in different lipoproteins

Although SM is not absorbed intact and the absorbed ceramide or sphingosine is not used extensively for the synthesis of chylomicron SM, there is growing interest in the nutritional effects of SM and in factors that regulate the level of plasma SM. It is known that the amount of phospholipids varies with different species (80). Compared with most other species (**Table 1**), humans have a high plasma SM level (~416 μg/ml), accounting for ~20% of the total plasma phospholipids (81). Animals with high HDL and low LDL levels, such as dog and rat, have rather high SM levels but a lower SM/PC ratio than humans. Low levels of plasma phospholipids and SM are

TABLE 1. Concentration of plasma phospholipids in different species

Species	Total Phospholipid	SM	PC	PC/SM	PE	LPC	PI
	mg/ml				*μg/ml*		
Human	2.22	416	1,558	3.7	66	168	NA
Cat	1.54	225	1,067	4.7	20	222	5
Dog	2.71	171	2,238	13.1	35	211	51
Pig	1.71	231	1,195	5.2	32	203	48
Rat	1.22	94	780	8.3	16	277	52
Cow	1.32	197	983	5.0	16	111	15
Goat	0.99	124	769	6.2	8	86	3
Sheep	0.75	101	538	5.3	8	101	4
Horse	1.27	135	951	7.0	42	137	4
Rabbit	1.23	82	748	9.1	82	250	68
Guinea pig	0.22	17	123	7.2	48	31	2

NA, not available; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, Phosphatidylethanolamine; PI, phosphatidylinositol; SM, sphingomyelin. Human data are calculated from Dougherty et al. (81) and animal data from Nelson (80).

found in herbivores such as guinea pig and rabbit and in chicken (e.g., plasma SM in the guinea pig amounts to only ~5% of the values found in humans). In the plasma, SM is present mainly in different lipoproteins. In human VLDL, SM accounts for ~12% of the phospholipids, in LDL plus VLDL it accounts for 28%, and in HDL it accounts for ~18%. The SM/PC ratio is lowest in HDL_2 and is 2-fold higher in LDL than in VLDL (82). A total of 63–75% of plasma SM is in LDL and VLDL and 25–37% is in HDL (83).

SM has amphiphilic properties similar to the most abundant phospholipid in plasma lipoproteins, PC. Because of the strong van der Waal interactions between SM and unesterified cholesterol (84–86), these lipids are associated in the polar surface coat. In the LDL particle, approximately two-thirds of the unesterified cholesterol is located in a mixed monolayer of PC and SM at the surface (87). ApoB influences the mobility of the polar head groups of the amphiphilic lipids. A study using ^{1}H NMR indicates that the polar head groups of part of the PC are immobilized as a result of interaction with apoB, whereas SM does not exhibit this feature (88). The finding that LDL, VLDL, and intermediate density lipoprotein (IDL) aggregate and exhibit increased affinity for uptake by macrophages when exposed to SMase (10, 20, 89, 90) emphasizes the fundamental importance of SM for the normal structure and metabolism of these lipoprotein classes. Hydrolysis of SM in HDL increased the fluidity of surface lipids, in contrast to hydrolysis of PC (91), and the presence of SM in reconstituted discoidal and spherical HDL was shown to increase PC acyl chain but not polar head group packing order and to inhibit the unfolding of apoA-I (92).

SM thus occurs in all lipoprotein classes and has important roles as an amphiphilic surface component with certain specific properties. Being resistant to lipases and LCAT and participating in spontaneous and phospholipid transfer protein (PLTP)-mediated transfer between lipoproteins, its key function may be to preserve particle stability during the wide range of lipoprotein particle modifications that are linked to TG and cholesterol transport. Although SMases are known to be present in blood, their quantitative capacity to metabolize lipoprotein SM has not been established.

Secretion of SM in TG-rich lipoproteins

SM is secreted into blood mainly in chylomicrons and VLDL. On an ordinary Western diet, 3–6 g of phospholipids enters blood via intestinal lipoproteins every day (93), of which ~7% is SM (26). The daily input of chyle SM into blood is then on the order of 0.3–0.4 g/day. In nascent VLDL, SM accounts for ~5% of the polar lipids (94). If nascent VLDL contains 10% polar lipids, of which 5% is SM, 0.1 g of SM per 20 g of VLDL is secreted. Recent stable isotope kinetic studies of VLDL TG production indicate a fasting production rate of 5–46 g/day (95), or 8–30 g of $VLDL_1$ and 0.7–4.6 g of $VLDL_2$ per day (96). The average production rate of VLDL apoB-100 was 14 mg/kg/day and 23 mg/day in the nonfasting state (97). Although the relation between apoB, TG, and SM secretion is not known, SM secretion is expected to exhibit a postprandial increase. The amount of SM secreted into blood as chylomicrons and VLDL should then be on the order of 0.5 g/day [i.e., approximately one-half plasma SM pool per day (24 h)]. It is expected to be higher in obese individuals having high VLDL secretion. Some SM is also secreted in discoidal HDL by liver and intestine (98). This secretion may involve the ABCA1 transporter, which was shown to be important by lipidating apoA-I in the liver (99). The amount of SM secreted into blood by these pathways is unknown, but it is likely to be smaller than the secretion in TG-rich lipoproteins.

Although SM is a component of nascent VLDL and chylomicrons, there is no evidence that it is essential for the secretory process. Hepatocytes secrete newly synthesized SM and ceramide in VLDL, but inhibition of SM and ceramide synthesis by the fungal toxin fumonisin did not inhibit VLDL secretion (100). The assembly of VLDL is initiated in the endoplasmic reticulum (ER), where apoB-100 has to be translocated to the lumen of the ER. ApoB-100 will interact with the lipids during this process, and the mature lipoprotein enters the secretory pathway when apoB-100 synthesis is completed and leaves the ribosome.

The assembly process is dependent on the active formation of PC and is highly dependent on the rate of TG synthesis and on the action of the microsomal TG transfer protein (101). Of the enzymes necessary for SM biosynthesis, palmitoyl-CoA-serine transferase and ceramide synthetase are located primarily in the ER. In contrast, the SM synthetase catalyzing the transfer of phosphocholine from PC to ceramide occurs in the Golgi vesicles and in the plasma membrane, in different isoforms (102). An intracellular protein that may transport ceramide to the site of SM synthesis has been characterized (103, 104). However, the regulation of VLDL SM secretion is poorly characterized and may occur at different synthetic steps as well as during the intracellular transfer of SM and ceramide, and SM might even be formed from lipoprotein ceramide after secretion.

The chylomicron surface coat contains apoA-I as a predominant apolipoprotein. The output of phospholipids increases after fat feeding, the phospholipid/apoA-I ratio being significantly higher than in circulating HDL. It is not known, however, how the ingestion of fat and cholesterol specifically influences SM secretion in chyle lipoproteins. An optimal chylomicron secretion depends on the formation of PC by reacylation of absorbed lysophosphatidylcholine and de novo synthesis, which increases with fat feeding. The role of SM, unesterified cholesterol, and apoA-I in the secretion of chylomicrons is not well known. In chyle, the proportion of apoA-I found in chylomicrons increases with lipid feeding, but the secretion in denser lipoproteins, including HDL, continues during fasting (93). Even bile diversion, which depletes rat intestinal epithelial cells and mesenteric lymph of TG-rich lipoproteins, does not decrease mesenteric apoA-I and HDL output (105). The fact that the polar surface material of chylomicrons has a composition like that of the material released from many cell types by active ABCA1 transporter-mediated transfer raises the question of whether this transporter may contribute PC, SM, and unesterified cholesterol during chylomicron formation. No analysis is available on chyle lipoproteins from patients with Tangier disease, which have a nonfunctioning ABCA1 transporter, or from ABCA1$^{-/-}$ mice, but both have decreased plasma TG values, primarily because of the rapid clearance of apoB-containing lipoproteins (106, 107). The ABCA1 transporter is present in the Golgi membrane of intestinal epithelial cells and transfers lipids from Golgi structures to the plasma membrane (108). The hypothesis that this localization is linked to a role in determining chylomicron composition and possibly in hepatic VLDL formation (109) is intriguing but unproven. Redgrave (110) found that Golgi "prechylomicrons" contain fewer phospholipids than the secreted particles, indicating that some are indeed added at a late stage.

SM in HDL is derived from chylomicron and VLDL secretion but also from peripheral tissues. The SM of VLDL and chylomicrons contains 16:0 and 18:0 as predominant fatty acids and fewer 20–24 carbon fatty acids than tissue SM (26, 111). If SM in LDL is derived primarily from VLDL and/or chylomicrons, one would expect a similar fatty acid composition, which was found to be the case (112). In HDL, a difference was noticed between HDL_2 and the denser HDL_3, which contains less phospholipid and cholesteryl ester (CE) and more protein than HDL_2. Whereas the fatty acid composition of SM in HDL_2 is similar to that of LDL, HDL_3 contains a larger proportion of 20–24 carbon fatty acid species. This suggests that HDL_2 contains relatively more polar surface phospholipids from VLDL and chylomicrons and HDL_3 relatively more tissue-derived SM, which contains more 20–24 carbon fatty acids. An alternative explanation is that there is a selective metabolism of different molecular species of HDL SM.

The fatty acid composition of plasma SM is rather stable. Myher et al. (113) fed diets rich in saturated and polyunsaturated fat to healthy young volunteers for 5 weeks. These conditions, which were sufficient to change the PC fatty acid pattern considerably, caused few changes in the fatty acid composition of SM.

In conclusion, chylomicron and VLDL secretion are major sources of plasma SM, although the mechanisms by which SM is inserted into these lipoproteins have not been established.

TRANSFER OF SM FROM CELLS TO LIPOPROTEINS

As shown in **Fig. 2**, plasma lipoprotein SM is derived from three sources: the intestine, the liver, and the tissue cells. Cells in tissues contain significant amounts of SM, much of which is colocalized with cholesterol in outer leaflets of plasma membranes, which are exposed to lipoproteins in blood or lymph. The transfer of cholesterol and SM between cells and lipoproteins involves both passive facilitated transfer mechanisms and energy-dependent ABCA transporter-mediated efflux.

Much of the direct contact between lipoproteins and cells occurs in the extracellular space. Therefore, it is interesting that lipoproteins in peripheral lymph, reflecting the composition of extracellular fluid, contain relatively more SM than their plasma counterparts (114). They may thus have acquired more SM from cell membranes or relatively less PC from TG-rich lipoproteins. Human peripheral lymph had a higher SM/PC ratio than plasma (115) and was found to contain small HDLs with a high level of SM (116). Infusion of apoA-I/PC discs increases the concentration of preβ-migrating HDL in tissue fluid and stimulates reverse cholesterol transport, but it is not known whether this is linked to an increased cellular efflux of SM (117).

In vitro, PC and SM but not phosphatidylethanolamine (PE) are exchanged between plasma lipoproteins and erythrocytes (118), and there is evidence also from other cell types that SM may be released to HDL by non-energy-dependent exchange/transfer, analogous to cholesterol (119) (Fig. 2). Partitioning of SM between lipoproteins and the erythrocytes by this pathway may be influenced by plasma lipoprotein concentration and composition. For example, in type II hypercholesterolemia, the SM/PC ratio of erythrocytes increases in parallel with an increase

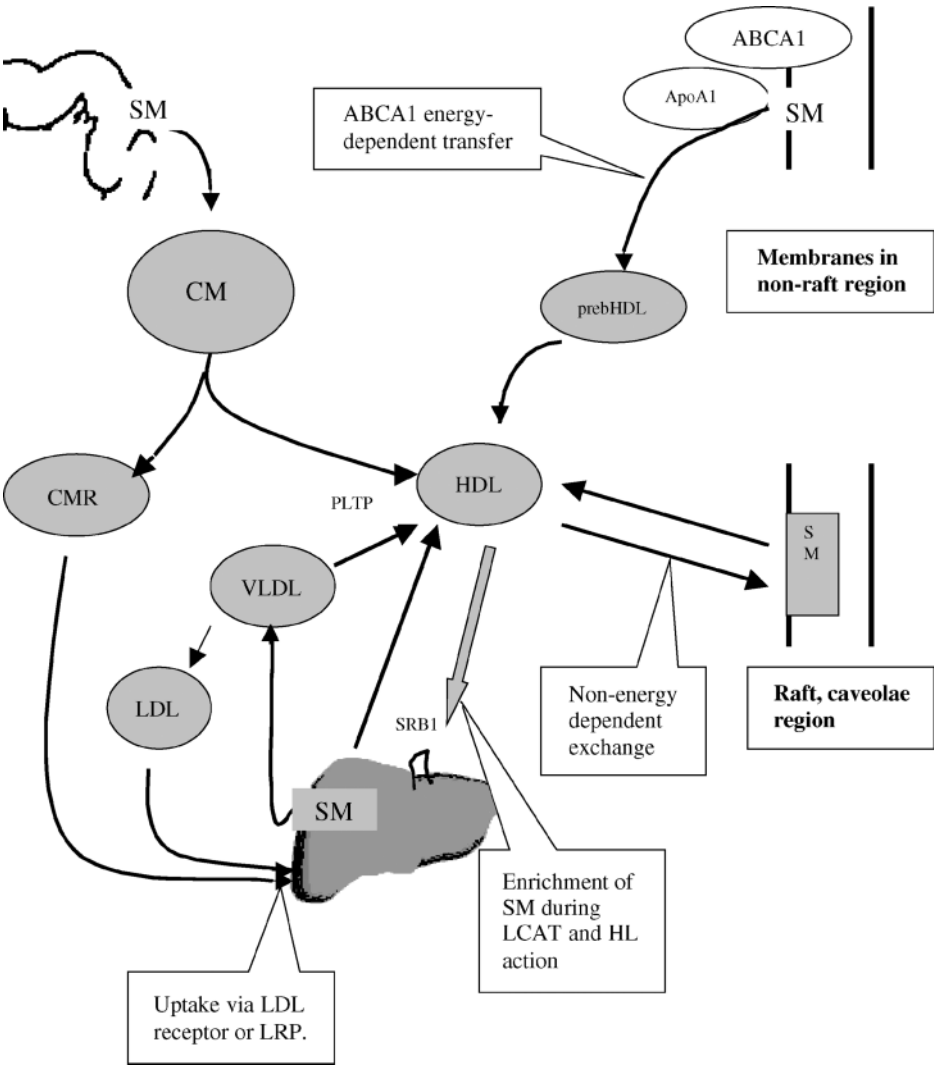

Fig. 2. Role of HDL in the transport of SM. HDL containing apolipoprotein A-I (apoA-I) as well as SM is secreted from both intestine and liver, where the ABCA1 transporter may participate in the lipidation. Some of the SM is transferred with the polar surface from chylomicrons (CM) and VLDL to HDL. In tissues, preβ-HDL acts as an acceptor for lipids in the energy-dependent transfer of lipids to HDL. Generally, both this material and that received from the polar surface of CM and VLDL have lower SM/phosphatidylcholine (PC) ratios than the circulating HDL. SM is thus enriched in HDL during its transformation by LCAT and HL. SM is also present in chylomicron remnants (CMR) and in intermediate density lipoprotein (IDL) and LDL formed from VLDL, as further described for Fig. 3. LRP, low density lipoprotein receptor-related protein; PLTP, phospholipid transfer protein; SR-BI, scavenger receptor class B type I.

in the SM/PC ratio of the plasma lipoproteins. The rapid normalization after apheresis treatment of severe type II hyperlipidemia suggests a rather rapid and concentration-dependent partitioning equilibrium of SM between plasma lipoproteins and the erythrocyte membrane (120). Whether similar equilibria influence the SM level of other cell types is not known. For instance, it is not known whether the increased SM/PC ratio in erythrocytes and in the adipocyte plasma membrane, which is found in obese patients and which correlates to insulin resistance (121, 122), is linked to an increased transfer of SM from lipoproteins to cells. The exchange/transfer of SM between cells and HDL may occur mainly in rafts and/or the caveolar region and is not mediated by the ABCA1 transporter. The quantitative importance of this transfer in vivo is not known but may be large.

It is well known that apoA-I releases both cholesterol and SM from cells (123, 124), and there is compelling evidence that this transfer involves the ABCA1 transporter. Preβ-migrating HDL, which is high in apoA-I and low in lipids, is a key acceptor during the active efflux of cholesterol and polar lipids, including SM (99, 125) (Fig. 2). An alternative cholesterol acceptor is the apoE-containing small SM-rich preγ-migrating HDL (92). The ABCA1 transporter, which is known to be important in cholesterol efflux from cells to lipoproteins, also transfers polar lipids, including SM, to apoA-I, occurring in monomolecular form or as poorly lipidated oliogomeric aggregates

(125–127) (Fig. 2). The transfer of SM is not selective, however, because the proportion of SM in lipoprotein complexes formed by ABCA1 activity (128) is generally lower than that of PC. The proportion of SM in the released material is also lower than in the acceptor lipoproteins [i.e., preβ-migrating HDL (129) and γ-lipoprotein E, which is rich in SM and contains apoE as the only apolipoprotein (130, 131)]. In some cell types, such as J774 macrophages, material released via ABCA1 activation contains a rather high proportion of SM among polar lipids (132).

Because loading of cells with cholesterol is known to increase ABCA1-associated cholesterol release to HDL, the question has been raised whether it also increases the proportion of SM in the released material. Bielicki, McCall, and Forte (133) showed that the differentiation of monocytes to macrophages increased SM content in the nascent HDL produced, but this did not increase further in foam cells produced by loading macrophages with acetylated LDL. Gillotte-Taylor et al. (134) enriched fibroblasts with cholesterol by incubating the cells with LDL or lipid/cholesterol dispersions. A doubling of the plasma membrane cholesterol was associated with a 6-fold increase in ABCA1-mediated efflux to apoA-I of both cholesterol and phospholipids, but the proportion of SM in the released material was decreased in cholesterol-loaded cells, and cholesterol loading increased PC rather than SM synthesis. Cholesterol excess in fibroblasts thus increased ABCA1-mediated SM efflux, but less than the cholesterol and PC efflux. Cholesterol loading of J744 macrophages increased the ABCA1-mediated cholesterol efflux and the cholesterol-phospholipid ratio of the released material severalfold but did not increase the proportion of SM in polar lipids (132).

The ABCA1 transporter is located in nonraft regions of the cells, and its function involves endocytosis and the resecretion of apoA-I (135). When lipidating an extracellular apolipoprotein acceptor, the transporter was shown to migrate between late endocytic vesicles and the cell surface, indicating that these vesicles play a role in cellular lipid efflux. In Tangier disease cells, both cholesterol and SM are accumulated, demonstrating a role of the ABCA1 transporter in the transport of SM. This model may explain why a deficient acid SMase decreases HDL (136), probably as a result of a defect of lipid efflux secondary to the inhibition of SM hydrolysis by acid lysosomal SMase (137, 138). It also supports the conclusion, drawn earlier by Mendez et al. (139), that cholesterol efflux to lipidated HDL particles but not the ABCA1-dependent efflux to lipid-free apoA-I involves the rafts. This was based on fibroblast studies showing that cholesterol efflux to HDL or plasma but not the ABCA1-mediated cholesterol efflux to apoA-I was associated with a reduction in raft cholesterol. Liu et al. (132), using J774 macrophages, demonstrated the apoA-I-induced release of apoA-I-containing particles, which were 9 and 12 nm compared with 6 nm for the lipid-free apoA-I and with a PC/SM ratio of 2:1. Furthermore, larger particles likely to be membrane vesicles, with a PC/SM ratio of 1:1, were formed. The conclusion was that the lipids of the apoA-I-containing particles were derived from nonraft regions, whereas the vesicles may be formed from cholesterol- and SM-rich lipid rafts. If so, ABCA1 may mediate the efflux of SM-rich lipid material in macrophages by two different mechanisms.

When another transporter, ABCA7, which is highly homologous to ABCA1, was overexpressed in HEK 293 cells, the apoA-I-mediated efflux of PC and SM, but not that of cholesterol, was increased (140).

In conclusion, SM may be released to HDL via the ABCA1 and possibly the ABCA7 transporter, but in much smaller amounts than PC. The quantity of SM supplied to HDL via these pathways is probably much less than the amount secreted in TG-rich lipoproteins and is weakly related to the reverse cholesterol transport. Little is known about whether a net transfer of SM from tissues to LDL, IDL, and/or VLDL occurs and whether HDL acceptors forward SM to LDL (115).

CAUSES OF HYPERSPHINGOMYELINEMIA

Both an increased VLDL production and a delayed clearance of remnants or LDLs increase SM levels. Patients with type II hyperlipidemia have increased plasma SM levels and increased proportions of SM in polar lipoprotein lipids (141). Jadhav and Thomson (142) concluded that there is both an increase in SM and a decrease in PC in the LDL particles. The increase in SM was related to an increased ratio of free to esterified cholesterol in LDL. The changes were in part reversed after LDL apheresis, when newly produced LDLs predominate, indicating that they are linked to prolonged residence time in plasma for the LDL particles (120, 142). The few data on type III human hyperlipidemia also reveal that the accumulation of remnant particles is linked to an increase in SM level in the lipoproteins (143). In contrast, the lipoprotein lipase-deficient type I hyperlipidemia is characterized by an increase in the PC/SM ratio. For other classes of hyperlipidemia, there is a correlation between cholesterol and SM levels but no decrease in the PC/SM ratio (144).

SM levels are also increased in hyperlipidemia animal models induced by diet or genetic defect. In the cholesterol-fed rabbit and in the hyperlipidemic LDL receptor-deficient WHHL rabbit, severalfold increases in plasma SM in lipoproteins are seen (145–147). In the apoE$^{-/-}$ knockout mouse, SM-rich lipoproteins, primarily cholesterol- and TG-rich remnants, accumulate (7). Whereas the WHHL rabbit and the apoE$^{-/-}$ mouse are characterized by a slow lipoprotein clearance attributable to a defective lipoprotein receptor-lipoprotein interaction, the SMHL rabbit is characterized by increased VLDL production. This rabbit also develops a moderate increase in plasma SM (148).

In chicken treated with ethinylestradiol, which drastically increases the secretion of VLDL TG, the plasma SM increases in parallel with TG (149), emphasizing that a high rate of VLDL secretion is an alternative cause of a high plasma SM. Because in this animal model VLDL that

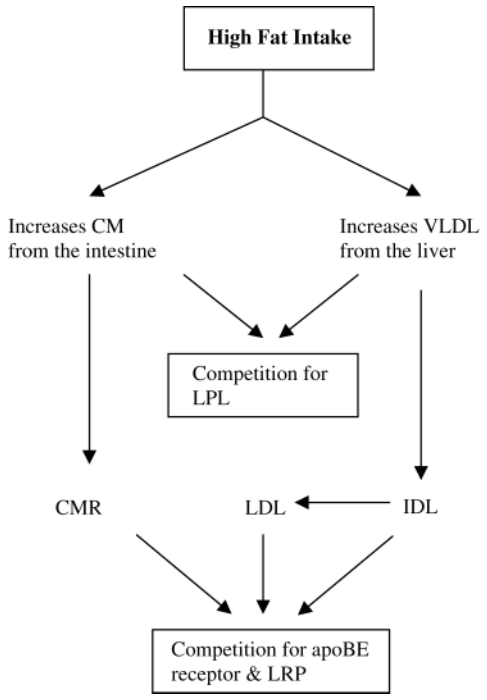

Fig. 3. Effects of a large lipid load on the metabolism of SM in apoB-containing lipoproteins. A large fat load leads to a high production of both chylomicrons (CM) and VLDL during the postprandial phase. CM, VLDL, and their partially lipolyzed remnants (CMR) will complete for LPL and apoE-dependent clearance via the apoB/E receptor and LRP. Postulating that the fractional turnover for SM in the triglyceride-rich particles is slower than for PC, the SM/PC ratio of the particles will increase with residence time. An increased residence time for IDL may also increase the partitioning to further interconversion to LDL rather than receptor-mediated catabolism.

is relatively resistant to LPL and specifically targeted to the egg is formed, a decreased clearance rate of VLDL may also contribute to the hypersphingomyelinemia (150).

Suckling neonates of all mammalian species ingest large amounts of milk lipids at frequent intervals. Suckling rats have 10-fold higher levels of LDL than normal adult rats and also high levels of lipoproteins in the LDL-VLDL area containing relatively large amounts of SM (111). During the neonatal phase, the rat expresses LPL in liver but has little adipose tissue at birth. Fatty acids are thus canalized to the liver for ketogenesis and VLDL production during postnatal growth, when adipogenesis is prominent in the rat (151). The findings in suckling rats must reflect both a high rate of chylomicron formation and an extensive recirculation of fatty acids as VLDLs.

Schlitt et al. (152) recently reported a postprandial increase in SM after ingestion of a lipid-rich meal (102 g, of which 52 g was saturated fat and 300 mg of cholesterol) that correlated to the postprandial increase in TG. Part of the increase in SM was in chylomicron remnant particles. Thus, also in adult humans, a large load of milk fat can induce postprandial hypersphingomyelinemia as a consequence of a high rate of chylomicron and VLDL production.

The consequences of a high-fat load for SM metabolism are summarized in **Fig. 3**. In this situation, increased amounts of chylomicrons and VLDLs and their remnants will compete for LPL and for apoB/E receptor- or low density lipoprotein receptor-related protein (LRP)-mediated elimination from plasma. As a consequence, the residence time of both types of intermediary TG-rich lipoproteins will increase. If one postulates that the fractional disappearance rate of SM in the TG-rich particles is lower than for PC, the SM/PC ratio will increase with the time needed for the particles to undergo lipolysis by LPL, clearance by receptors as remnants, or conversion to LDL. SM in plasma may be linked to the rate of VLDL production, which may explain why in the study by Schlitt et al. (152) the clearance of postprandial remnant-like particles was related to serum SM level. Using this model, one expects plasma SM to increase in situations with an exaggerated postprandial lipemic response as combined hyperlipidemia (153) and obesity (154). The number of VLDL particles clearly exceeds the number of chylomicrons, even during the postprandial phase, but still chylomicrons strongly inhibit VDL clearance, possibly as a result of their high affinity for LPL (155). Therefore, the effect on SM accumulation may be strongest in VLDL remnants. This model for the influence of large dietary fat loads on plasma SM does not need to postulate that the inhibitory action of SM on LPL and on apoE-mediated remnant clearance, described below and in **Table 2**, occurs in vivo. If it does, it will influence the clearance of those particles that have the lowest affinity for LPL and for apoE-mediated clearance most markedly.

Thus, hypersphingomyelinemia is strongly related to the production and clearance of apoB-containing lipo-

TABLE 2. Effects of SM in lipoproteins

Location of SM	Effects	References
Chylomicrons	Inhibits TG hydrolysis by LPL	185–190
Chylomicron remnants	Inhibits receptor-mediated metabolism of CE	185–190
VLDL	Inhibits TG hydrolysis by LPL	152
VLDL	Enhances apoE loss to HDL	191–193
IDL	Enhances apoE loss to HDL	191–193
IDL	Inhibits receptor-mediated metabolism of CE	193
LDL	Inhibits binding of LDL to LDL receptor	182
LDL	Inhibits binding to macrophage scavenger receptors	10, 20
HDL	Inhibits LCAT and CE formation	92, 196
HDL	Inhibits scavenger receptor class B type I-mediated uptake of CE	201

apoE, apolipoprotein E; CE, cholesteryl ester; IDL, intermediate density lipoprotein; TG, triglyceride. The table summarizes the effects of SM found in vitro and/or in experiments with lipid emulsions in vivo. SM inhibits the action of LPL and decreases the binding of apoE to TG-rich lipid or lipoprotein particles. These effects may be particularly important in situations in which a large number of TG-rich lipoprotein particles with varying affinity for these pathways compete. Thus, they may enhance the effects of the high lipid intake illustrated in Fig. 3. SM also inhibits LCAT by decreasing the affinity of the enzyme for HDL particles. LCAT may thereby prefer PC-rich HDL for its action and set a limit for the degree of HDL particle transformation that can be achieved by LCAT action.

proteins. It reflects the accumulation of particles that have undergone different stages of lipolysis. It can be seen as an adaptation to increased transport demands on these lipoproteins.

METABOLISM OF PLASMA SM

Chylomicron and VLDL SM

During the metabolism of chylomicron TG by LPL, most of the polar lipids are released as vesicular structures (156–158) and are transferred to HDL by the action of PLTP (159, 160), where PE and PC are metabolized by the action of hepatic lipase and LCAT, which do not metabolize SM (161). LPL may also act on glycerophospholipids in chylomicrons (162). How the relative rates of transfer of individual phospholipids are influenced by lipase and PLTP action in humans is unknown. Minari and Zilversmit (163) incubated ^{32}P-labeled dog chyle chylomicrons with serum and found a net loss of phospholipids to HDL, which was accounted for by PC and PE. SM exchanged in chylomicrons with other lipoproteins, but the mass in the chylomicrons changed little. Another early study found a larger proportion of SM in plasma chylomicrons than in chyle chylomicrons in humans, 12% versus 7% of the polar lipids (164), and in rat the proportion of SM is higher in plasma VLDL than in nascent VLDL (94, 165). Whether this difference should be attributed to the action of lipases or differences in kinetics for the transfer of different polar lipids between lipoprotein classes is not known, although PLTP mediates the transfer of SM as well as glycerophospholipids (159, 166). When VLDL was incubated with LPL, the level of γ-migrating lipoprotein E increased, suggesting that these particles may be formed from surface material during lipolysis (167).

Most chylomicron remnants are rapidly catabolized, primarily by the apoE-mediated action of the apoB/E receptor and the LRP receptor in the liver (168). In the case of VLDL, which contains both apoE and apoB when secreted in nascent form, some remnants formed during lipase and PLTP action are cleared and some are transformed to IDL and finally LDL with a slow turnover (97, 169). What regulates the SM content of these SM-rich atherogenic lipoproteins is important but not known. In most species, detailed information on the SM/apoB ratio during the transformation of VLDL to IDL and LDL is lacking. Thus, it is unknown whether in humans SM is enriched by the continuous lipolysis of other lipids only or by the net transfer of SM during the exchange with other lipoproteins as well. Interestingly, recent data on the phospholipid/apoB ratio and the percentage of SM in polar lipids in VLDL, IDL, and LDL (148) in the WHHL, the SMHL, and the normal New Zeeland White rabbit indicate that the SM/apoB ratios in VLDL, IDL, and LDL continuously decrease during the transformation of VLDL to IDL and LDL. The ratio in VLDL and LDL decreased from 0.57 to 0.06 in the SMHL rabbit, from 0.13 to 0.08 in normal rabbits, and from 1.07 to 0.43 in the WHHL rabbit. Thus, there was no evidence of a net acquisition of SM from LDL but a continuous loss of SM. As a consequence, any receptor-mediated catabolism of intermediary particles during the VLDL-LDL interconversion will eliminate relatively more SM per lipoprotein particle than the LDL catabolism.

SM is transferred between different lipoprotein classes

Early studies demonstrated an exchange of SM between VLDL, LDL, and HDL (170). This bidirectional transfer may be both spontaneous and mediated by PLTP (92). Rao et al. (166) studied the molecular and macromolecular specificity of human PLTP and found that the most rapid transfer of phospholipids occurred between different HDL subfractions. The spontaneous transfer of SM was faster than that of PC, and the PLTP-catalyzed transfer was slower. In studies of the action of PLTP and HL on HDL_2, denser SM-rich particles were formed as a minority of the particles (171). Therefore, one can postulate that during the continuous transformation of TG-rich lipoproteins to remnants and LDL, there is a partitioning of SM that depends on available SM mass as well as LDL, HDL, and remnant composition. The factors that regulate this partitioning in vivo need to be characterized.

METABOLISM OF SM IN LDL AND HDL

Because the plasma SM/PC ratio is approximately three times greater than in secreted TG-rich lipoproteins, the fractional turnover of SM must be approximately one-third of that for glycerophospholipids, primarily PC. Studies by Zilversmit, Entenman, and Chaikoff (172, 173) in 1948, using ^{32}P isoptope kinetics and infusion of ^{32}P-labeled plasma in dogs (mainly HDL), demonstrated a longer turnover time for plasma SM (up to 27 h) than for plasma PC (up to 13 h). Generally, however, studies of lipoprotein SM metabolism in vivo are few, and the quantitative contributions by the different potential pathways are poorly characterized. The alternatives are that SM carried in HDL, IDL, and LDL may be metabolized by particulate metabolism of the whole lipoprotein or by lipid transfer to cells primarily via the scavenger receptor class B type I (SR-BI).

Bentejac et al. (174) incorporated [^{14}C]stearate- plus [^{3}H]choline-labeled SM into HDL in vitro and studied the fate of the doubly labeled lipoprotein SM in vivo in rats. The decay of plasma radioactivity followed a triexponential curve with one fast component, one intermediary with a half-life of ~100 min, and a slow component. Tissue radioactivity was examined after 24 h and was greatest per gram of tissue in liver and adrenals. Studies from the same group also compared the fate of 18:0 and 24:0 SM and found only small differences (175, 176). Stein et al. (177) injected unilamellar liposomes of SM with defined fatty acids and labeled the 18:0, 18:1, or 18:2 fatty acid intravenously and found evidence for some reutilization in the liver of palmitoyl-sphingosine but not of linoleoyl-sphingosine for SM synthesis. This conclusion was supported in

experiments with [^{3}H]sphingosine-, [^{14}C]palmitic acid-, and [^{14}C]linoleic acid-labeled SM in vivo and in HepG2 cells (178).

The conclusion from these studies is that SM is cleared from plasma faster than apoA-I of HDL. These results are compatible with some uptake by the SR-BI receptor but at a slower rate than HDL CE. The ability of SR-BI to mediate the cellular uptake of SM from HDL has actually been demonstrated in COS-7 cells (179) and monocytes (180) using LDL, HDL, and lipid vesicles as carriers of pyrene-labeled phospholipids. Overexpression of SR-BI in BHK cells was found to increase the uptake of LDL SM most. Interestingly, overexpression of SR-BI in apoE$^{-/-}$ mice, which accumulate SM-rich lipoproteins, did not improve the defective lipoprotein clearance of apoB-containing lipoproteins in these animals (181). The conclusion is that SR-BI participates in the clearance of SM from HDL and possibly also from LDL, but the uptake is less efficient than the SR-BI-mediated uptake of CE.

Studies in cell cultures indicate that LDL SM can be degraded after uptake of the lipoprotein particle via the LDL receptor (182). SMase treatment of LDL caused an enhanced uptake by cultured cells, at least in part mediated by the LDL receptor (9). Evidence was provided that in lymphoblastoid cells, LDL SM could be processed by both an endosomal pathway involving lysosomal SMase and an alternative pathway by which LDL SM was degraded by neutral SMase (183). Interestingly, the uptake of LDL by fibroblast cultures was also found to be linked to the feedback suppression of sphinganine biosynthesis (184).

Studies of the clearance of HDL and LDL SM in humans are lacking. An approximate calculation from known data on apoA-I turnover in humans (~0.20–0.25 pools/d) and a total plasma HDL SM pool of ~0.3–0.4 g indicates that one-third to one-fourth of the HDL SM can be eliminated by particulate metabolism of HDL. A similar calculation for LDL SM, postulating an average apoB turnover of 0.46 pools/day (97) and a total plasma LDL SM pool of 0.7–1 g, indicates that less than half of the LDL SM could be cleared from plasma via the elimination of LDL particles, including apoB.

Thus, particulate degradation of LDL and HDL may account for a part but not all of the plasma SM turnover. Both the SR-BI receptor and lipoprotein receptor-mediated uptake of chylomicron remnant particles and VLDL remnants are likely to contribute, but the quantitative importance of these pathways needs to be defined. Clearly, the fractional turnover of HDL SM is lower than for HDL-PC, the SM/PC ratio being higher in circulating HDL than in polar lipids received from chylomicrons, VLDL, and the ABCA1 transporter.

FUNCTIONS OF SM IN LIPOPROTEINS

SM inhibits TG hydrolysis by LPL in chylomicron and VLDL

Several studies both in vivo and in vitro indicate that the presence of SM in chylomicron and VLDL may inhibit the action of LPL. Kuksis et al. (185) compared PC-TG, PC-SM-TG, and PC-PE-TG emulsions and found that PE-containing emulsion had the fastest clearance rate and PC-SM-TG had the slowest. Redgrave et al. (186) injected TG-rich emulsions and found that inclusion of 40% or more among the polar lipids significantly delayed particle removal. In vitro, Saito et al. (187) and Arimoto et al. (188) found that SM but not cholesterol significantly inhibited lipolysis by LPL. The SM/cholesterol ratio of the surface lipids may thus be an important determinant of lipolysis rate (188). Similar findings were made by Lobo and Wilton (189) and Cantin et al. (190), who showed that cholesterol inclusion into the PC surface coat of a TG emulsion stimulated lipolysis by LPL, but this stimulation was eliminated by adding 15% SM to the polar lipid mix.

SM decreases the association of apoE with remnants

In apoE$^{-/-}$ mice, SM-rich remnant particles accumulate (7). Because apoE has a key role in the rapid removal of remnant particles, the question has been asked how SM and apoE interact under normal conditions. Studies by Arimoto et al. (191) using artificial lipid emulsions with different SM content suggested that SM may delay remnant clearance by decreasing the binding of apoE, thereby decreasing interaction with the LRP and the apoB/E receptor. In artificial lipid emulsions of TG-PC-SM, apoE was found to prevent particle aggregation induced by SMase. Furthermore, particles consisting of triolein and a polar lipid mixture of PC/SM (2:1) were found to bind apoE less efficiently than an emulsion containing PC as the only polar lipid (192). The presence of ceramide in the emulsion with PC/SM (2:1) increased the binding. The incorporation of SM in the emulsion decreased the capacity for binding of apoE without changing affinity. ApoC-II and apoC-III inhibited the apoE-mediated cellular (HepG2) uptake of the particles more efficiently if SM was present in the particles (193).

Thus, there is evidence that SM inhibits both the action of LPL and the interaction of TG-rich particles with apoE. Although the in vivo relevance of these findings is not fully established, they may be highly relevant during the SM increase after a period of large lipid load (152).

SM inhibits LCAT but not cholesteryl ester transfer protein action

During reverse cholesterol transport, cholesterol is transferred from cells to HDL, where CE is formed by LCAT and metabolized primarily by the action of the SR-BI receptor in liver and steroidogenic tissues (194, 195) or transferred by the action of cholesteryl ester transfer protein (CETP) to LDL and TG-rich lipoproteins in exchange for TG. A number of studies indicate that this procedure is affected by SM in HDL. SM may inhibit LCAT by decreasing enzyme binding (196). A negative correlation between SM content and LCAT activity was observed in studies with proteoliposomes or reconstituted HDL (197). Rye, Hime, and Barter (92) found that SM influences the structure of discoidal and spherical HDL and

confirmed that SM inhibits the LCAT reaction but not CETP. The metabolism of lipoprotein SM may in turn be influenced by LCAT, because inhibition of LCAT minimized SM exchange among lipoproteins (170). ApoA-ISeattle, which is linked to a decrease in LCAT activity, recruited lipids with a higher proportion of SM and PE (198). In children with kwashiorkor, a decrease in LCAT action could be related to changes in HDL composition, including an increased proportion of SM (199).

Thus, there is no evidence that SM affects CETP function (92), and CETP and PLTP have no overlapping function in vivo. In PLTP knockout mice that were made transgenic for CETP, the phospholipid transfer was still zero (200). Thus, if more SM is partitioned to CE-rich particles in hypercholesterolemia, this has to occur by other mechanisms than a CETP-mediated cotransport with CE. Nor is there any evidence that SM influences the SR-BI-mediated uptake of CE. Generally, the uptake of CE in the liver is more effective for the largest HDL particles, which should indicate that it is less efficient for small SM-rich particles (201). A key function of plasma SM may be in retaining HDL structure during the transformation of HDL, which regenerates SM-rich subfractions of particles that are optimized as acceptors for cholesterol (171).

In summary (Table 2), the inhibitory effects of SM on LPL, LCAT, and apoE binding to lipoproteins are well established, but there is still uncertainty about their physiological importance in vivo in humans. After a large fat load, when TG-rich lipoproteins compete for the LPL- and apoE-mediated catabolism, an SM-mediated inhibitory effect on these catabolic pathways may be important both for the clearance of the most TG-rich lipoproteins and for the partitioning of liver-derived VLDL remnants between rapid apoE-mediated remnant catabolism and the formation of LDL with a slow turnover.

EFFECTS OF DIET AND AGE ON SM TRANSPORT

Effects of diet

There are relatively few studies on dietary effects on plasma SM. In cholesterol-fed rabbits, SM accumulates in the cholesterol-rich lipoproteins. This severalfold increase in plasma SM in the cholesterol-fed rabbit was early observed by McCandless and Zilversmit (202) and later confirmed by others (203). Using ^{32}P label, these authors also found that the rate of synthesis of plasma SM is increased significantly in the cholesterol-fed animals. Geelen et al. (204) reported an increase of plasma SM in rats fed cholesterol. Benade et al. (205) fed an atherogenic diet consisting entirely of normal foods to female vervet monkeys for 4 years as well as a more prudent control diet. The LDL cholesterol was 3-fold higher and the SM was 2-fold higher with the atherogenic diet as a result of the increased number of LDL particles. The LDL SM level correlated strongly to atherosclerosis measures and scores. Kruger et al. (206) fed a diet with different contents of saturated and polyunsaturated fat and total fat. Substantial variations in LDL SM levels but no great variations in particle composition were seen. Thus, in dietary experiments that are relevant to normal human diets, the plasma SM is primarily influenced by the number of LDL particles.

Parks and Gebre (207) compared a fish oil-enriched diet and a lard diet in cynomolgus monkeys and found the fish oil diet to decrease HDL without any change in absolute levels of LDL, apoB, and LDL cholesterol. SM, however, was increased in LDL and PC decreased after fish oil feeding. Geelen and Beynen (208) compared the effects of coconut oil and olive oil on lipoprotein cholesterol and SM levels in rats on a cholesterol-rich diet and noticed that plasma SM was lower in the olive oil-fed group, although VLDL SM was increased. Olive oil also reduced serine palmitoyltransferase (i.e., the enzyme catalyzing the first step in the synthesis of sphingoid bases) (208). Casein compared with soy protein fed to rats in a cholesterol-rich diet caused a transient increase in VLDL production as well as increased VLDL SM and decreased LDL and HDL SM (209). Bagdade et al. (210) reported an increase in HDL_2 including SM and a decrease in TG after feeding fish oil to women with insulin-dependent diabetes mellitus. Interestingly, a decrease of choline-containing polar lipids in apoB-carrying lipoproteins was reported in well-controlled insulin-treated diabetes mellitus (210, 211).

Effects of dietary sphingolipids

Recently, it was reported that feeding apoB/E receptor-deficient mice a sphingolipid-rich diet for 3 months increased non-HDL SM and non-HDL cholesterol significantly but not extensively (212). The degree of arteriosclerosis was increased. Kobayashi et al. (213), by feeding 1% sphingolipids to rats through two generations, observed a 30% decrease in plasma cholesterol and no change in plasma phospholipid classes, whereas a study over a shorter time period reported an increase in plasma SM (214). Although SM is generally not absorbed intact (28, 29) and SM feeding in lymphatic duct cannulation studies rather decreased lymphatic SM output (62), there are mechanisms other than the inhibition of cholesterol absorption by which dietary SM may influence plasma lipoprotein levels during more prolonged periods.

Effects of drugs

There is little information on plasma SM levels after treatment with lipid-lowering drugs. In rats, pectin, which decreased plasma and hepatic cholesterol, also decreased VLDL SM and increased HDL SM. Pectin also increased lysosomal acid as well as neutral plasma membrane SMase (215). In postmenopausal women, the combination of atorvastatin and the estrogen receptor modulator raloxifene was shown to decrease plasma phospholipids and increase the PC/SM ratio of HDL. Atorvastatin alone did not significantly decrease serum HDL SM (216). After a 10–12 week treatment with 10 mg of simvastatin per day, providing an average decrease of plasma cholesterol from 277 to 210 mg/dl (217), a moderate decrease was observed in the proportion of SM in polar HDL lipids, with the proportions of PC and PE increasing.

Recently, the use of the SM synthesis inhibitor myriocin in apoE$^{-/-}$ mice demonstrated that plasma SM could be decreased without significant reduction of cholesterol and TG levels (19, 218). Notably, the degree of arteriosclerosis was decreased in both studies. Although theoretically, the positive effect on arteriosclerosis could be related either to inhibition of SM synthesis in the arterial wall or in liver and intestine, or to other mechanisms, these findings strengthen the idea that the SM content of atherogenic lipoproteins is important in atherogenesis.

Effects of age

The proportion of SM in plasma lipoproteins increases with age in both children and adults (219, 220). The mechanism behind this change with age has not been clarified. A delayed clearance of LDL, decreased food intake, and thus a decreased transfer of polar surface material from chylomicrons and VLDL may contribute. There is also an increase in cellular SM with age, which was suggested to have a role for SM metabolites in life span regulation (221). Interestingly, there is a paradoxical decrease in the oxidizability of LDL with increasing age. SM is not very susceptible to oxidation because of its low content of polyunsaturated fatty acids, and it also may protect other lipids from oxidation. For example, Subbaiah, Subramanian, and Wang (222) found that the susceptibility of LDL to oxidation in vitro was inversely related to the content of SM.

CONCLUSIONS

Dietary SM is not absorbed intact but is metabolized to ceramide, phosphocholine, sphingosine, and fatty acids in the gut. There is evidence for an inhibitory effect on cholesterol absorption, an influence on lipoprotein secretion, and an antitumor effect, which may be mediated by the signaling effects of the SM metabolites and/or the interaction of SM with cocarcinogenic bile acids. These intestinal effects need to be further characterized. Secretion as chylomicrons and VLDL contributes a major part of the plasma lipoprotein, but the effects of diet and other factors that regulate SM secretion in these lipoproteins are poorly characterized. The enrichment of SM in atherogenic LDL and TG-rich remnant lipoproteins can be understood from the action of lipases and LCAT on glycerolipids but not on SM. The progressive increase of SM during the prolonged metabolism of lipoprotein particles (e.g., in type II hyperlipidemia and apoE deficiency) can be linked to multiple effects. During prolonged residence times, apoB-containing lipoproteins may become more dependent on SM to prevent aggregation, recognition by macrophage scavengers, and intravascular oxidation, but they also may be more vulnerable to SMase action in the arterial wall, resulting in particle aggregation, foam cell formation, and enhanced atherogenesis. The ability to enrich SM in TG-rich lipoproteins may also be viewed as an important physiological mechanism to expand the plasma lipid pools temporarily during periods of large lipid loads to optimize the targeting of fatty acids to tissues and avoid lipotoxic effects. In this context, the presence of an amphiphilic component, which is resistant to lipases and LCAT and moreover may inhibit the action of these enzymes, is rational from a teleological point of view. More information is needed on the role of the inhibitory effect of SM on LPL, LCAT, and lipoprotein-receptor interaction in vivo and on the relation between the SM and apoE transfer reactions, particularly during the continuous conversion of VLDL to LDL.

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REFERENCES

1. Slotte, J. P. 1999. Sphingomyelin-cholesterol interactions in biological and model membranes. *Chem. Phys. Lipids.* **102:** 13–27.
2. Ridgway, N. D. 2000. Interactions between metabolism and intracellular distribution of cholesterol and sphingomyelin. *Biochim. Biophys. Acta.* **1484:** 129–141.
3. Portman, O. W., and M. Alexander. 1970. Metabolism of sphingolipids by normal and atherosclerotic aorta of squirrel monkeys. *J. Lipid Res.* **11:** 23–30.
4. Portman, O. W., and D. R. Illingworth. 1976. Arterial metabolism in primates. *Primates Med.* **9:** 145–223.
5. Stein, O., S. Eisenberg, and Y. Stein. 1969. Aging of aortic smooth muscle cells in rats and rabbits. A morphologic and biochemical study. *Lab. Invest.* **21:** 386–397.
6. Jiang, X. C., F. Paultre, T. A. Pearson, R. G. Reed, C. K. Francis, M. Lin, L. Berglund, and A. R. Tall. 2000. Plasma sphingomyelin level as a risk factor for coronary artery disease. *Arterioscler. Thromb. Vasc. Biol.* **20:** 2614–2618.
7. Jeong, T., S. L. Schissel, I. Tabas, H. J. Pownall, A. R. Tall, and X. Jiang. 1998. Increased sphingomyelin content of plasma lipoproteins in apolipoprotein E knockout mice reflects combined production and catabolic defects and enhances reactivity with mammalian sphingomyelinase. *J. Clin. Invest.* **101:** 905–912.
8. Tabas, I., Y. Li, R. W. Brocia, S. W. Xu, T. L. Swenson, and K. J. Williams. 1993. Lipoprotein lipase and sphingomyelinase synergistically enhance the association of atherogenic lipoproteins with smooth muscle cells and extracellular matrix. A possible mechanism for low density lipoprotein and lipoprotein(a) retention and macrophage foam cell formation. *J. Biol. Chem.* **268:** 20419–20432.
9. Gupta, A. K., and H. Rudney. 1992. Sphingomyelinase treatment of low density lipoprotein and cultured cells results in enhanced processing of LDL which can be modulated by sphingomyelin. *J. Lipid Res.* **33:** 1741–1752.
10. Marathe, S., Y. Choi, A. R. Leventhal, and I. Tabas. 2000. Sphingomyelinase converts lipoproteins from apolipoprotein E knockout mice into potent inducers of macrophage foam cell formation. *Arterioscler. Thromb. Vasc. Biol.* **20:** 2607–2613.
11. Alessenko, A. V. 2000. The role of sphingomyelin cycle metabolites in transduction of signals of cell proliferation, differentiation and death. *Membr. Cell Biol.* **13:** 303–320.
12. Futerman, A. H., and Y. A. Hannun. 2004. The complex life of simple sphingolipids. *EMBO Rep.* **5:** 777–782.
13. Gulbins, E., and R. Kolesnick. 2003. Raft ceramide in molecular medicine. *Oncogene.* **22:** 7070–7077.
14. Hannun, Y. A., C. Luberto, and K. M. Argraves. 2001. Enzymes of sphingolipid metabolism: from modular to integrative signaling. *Biochemistry.* **40:** 4893–4903.
15. Hannun, Y. A., and C. Luberto. 2004. Lipid metabolism: ceramide transfer protein adds a new dimension. *Curr. Biol.* **14:** R163–R165.

16. Marchesini, N., and Y. A. Hannun. 2004. Acid and neutral sphingomyelinases: roles and mechanisms of regulation. *Biochem. Cell Biol.* **82:** 27–44.
17. Auge, N., A. Negre-Salvayre, R. Salvayre, and T. Levade. 2000. Sphingomyelin metabolites in vascular cell signaling and atherogenesis. *Prog. Lipid Res.* **39:** 207–229.
18. Park, T. S., R. L. Panek, S. B. Mueller, J. C. Hanselman, W. S. Rosebury, A. W. Robertson, E. K. Kindt, R. Homan, S. K. Karathanasis, and M. D. Rekhter. 2004. Inhibition of sphingomyelin synthesis reduces atherogenesis in apolipoprotein E-knockout mice. *Circulation.* **110:** 3465–3471.
19. Hojjati, M. R., Z. Li, H. Zhou, S. Tang, C. Huan, E. Ooi, S. Lu, and X. C. Jiang. 2005. Effect of myriocin on plasma sphingolipid metabolism and atherosclerosis in apoE-deficient mice. *J. Biol. Chem.* **280:** 10284–10289.
20. Tabas, I. 2004. Sphingolipids and atherosclerosis. A mechanistic connection? A therapeutic opportunity? *Circulation.* **110:** 3400–3401.
21. Vesper, H., E. M. Schmelz, M. N. Nikolova-Karakashian, D. L. Dillehay, D. V. Lynch, and A. H. Merrill, Jr. 1999. Sphingolipids in food and the emerging importance of sphingolipids to nutrition. *J. Nutr.* **129:** 1239–1250.
22. Zeisel, S. H., D. Char, and N. F. Sheard. 1986. Choline, phosphatidylcholine and sphingomyelin in human and bovine milk and infant formulas. *J. Nutr.* **116:** 50–58.
23. Zeisel, S. H., M. H. Mar, J. C. Howe, and J. M. Holden. 2003. Concentrations of choline-containing compounds and betaine in common foods. *J. Nutr.* **133:** 1302–1307.
24. Alvaro, D., A. Cantafora, A. F. Attili, S. Ginanni Corradini, C. De Luca, G. Minervini, A. Di Biase, and M. Angelico. 1986. Relationships between bile salts hydrophilicity and phospholipid composition in bile of various animal species. *Comp. Biochem. Physiol. B.* **83:** 551–554.
25. Nibbering, C. P., and M. C. Carey. 1999. Sphingomyelins of rat liver: biliary enrichment with molecular species containing 16:0 fatty acids as compared to canalicular-enriched plasma membranes. *J. Membr. Biol.* **167:** 165–171.
26. Zilversmit, D. B. 1968. The surface coat of chylomicrons: lipid chemistry. *J. Lipid Res.* **9:** 180–186.
27. Schlierf, C., W. H. Falor, P. D. Wood, Y. L. Lee, and L. W. Kinsell. 1969. Composition of human chyle chylomicrons following single fat feedings. *Am. J. Clin. Nutr.* **22:** 79–86.
28. Nilsson, A. 1968. Metabolism of sphingomyelin in the intestinal tract of the rat. *Biochim. Biophys. Acta.* **164:** 575–584.
29. Nilsson, A. 1969. The presence of sphingomyelin- and ceramide-cleaving enzymes in the small intestinal tract. *Biochim. Biophys. Acta.* **176:** 339–347.
30. Fukuda, Y., A. Kihara, and Y. Igarashi. 2003. Distribution of sphingosine kinase activity in mouse tissues: contribution of SPHK1. *Biochem. Biophys. Res. Commun.* **309:** 155–160.
31. van Veldhoven, P. P., and G. P. Mannaerts. 1993. Sphingosine-phosphate lyase. *Adv. Lipid Res.* **26:** 69–98.
32. Schmelz, E. M., K. J. Crall, R. Larocque, D. L. Dillehay, and A. H. Merrill, Jr. 1994. Uptake and metabolism of sphingolipids in isolated intestinal loops of mice. *J. Nutr.* **124:** 702–712.
33. Duan, R. D., E. Hertervig, L. Nyberg, T. Hauge, B. Sternby, J. Lillienau, A. Farooqi, and A. Nilsson. 1996. Distribution of alkaline sphingomyelinase activity in human beings and animals. Tissue and species differences. *Dig. Dis. Sci.* **41:** 1801–1806.
34. Duan, R. D., and A. Nilsson. 1997. Purification of a newly identified alkaline sphingomyelinase in human bile and effects of bile salts and phosphatidylcholine on enzyme activity. *Hepatology.* **26:** 823–830.
35. Cheng, Y., A. Nilsson, E. Tomquist, and R. D. Duan. 2002. Purification, characterization, and expression of rat intestinal alkaline sphingomyelinase. *J. Lipid Res.* **43:** 316–324.
36. Duan, R. D., Y. Cheng, G. Hansen, E. Hertervig, J. J. Liu, I. Syk, H. Sjostrom, and A. Nilsson. 2003. Purification, localization, and expression of human intestinal alkaline sphingomyelinase. *J. Lipid Res.* **44:** 1241–1250.
37. Duan, R. D., T. Bergman, N. Xu, J. Wu, Y. Cheng, J. Duan, S. Nelander, C. Palmberg, and A. Nilsson. 2003. Identification of human intestinal alkaline sphingomyelinase as a novel ecto-enzyme related to the nucleotide phosphodiesterase family. *J. Biol. Chem.* **278:** 38528–38536.
38. Wu, J., Y. Cheng, C. Palmberg, T. Bergman, A. Nilsson, and R. D. Duan. 2005. Cloning of alkaline sphingomyelinase from rat intestinal mucosa and adjusting of the hypothetical protein XP_221184 in GenBank. *Biochim. Biophys. Acta.* **1687:** 94–102.
39. Wu, J., F. Liu, A. Nilsson, and R. D. Duan. 2004. Pancreatic trypsin cleaves intestinal alkaline sphingomyelinase from mucosa and enhances the sphingomyelinase activity. *Am. J. Physiol. Gastrointest. Liver Physiol.* **287:** G967–G973.
40. Olsson, M., R. D. Duan, L. Ohlsson, and A. Nilsson. 2004. Rat intestinal ceramidase: purification, properties, and physiological relevance. *Am. J. Physiol. Gastrointest. Liver Physiol.* **287:** G929–G937.
41. Mitsutake, S., M. Tani, N. Okino, K. Mori, S. Ichinose, A. Omori, H. Iida, T. Nakamura, and M. Ito. 2001. Purification, characterization, molecular cloning, and subcellular distribution of neutral ceramidase of rat kidney. *J. Biol. Chem.* **276:** 26249–26259.
42. Choi, M. S., M. A. Anderson, Z. Zhang, D. B. Zimonjic, N. Popescu, and A. B. Mukherjee. 2003. Neutral ceramidase gene: role in regulating ceramide-induced apoptosis. *Gene.* **315:** 113–122.
43. 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.
44. Duan, R. D., Y. Cheng, L. Yang, L. Ohlsson, and A. Nilsson. 2001. Evidence for specific ceramidase present in the intestinal contents of rats and humans. *Lipids.* **36:** 807–812.
45. 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.
46. Nyberg, L., R. D. Duan, J. Axelsson, and Å. Nilsson. 1997. Localization and capacity of sphingomyelin digestion in the rat intestinal tract. *J. Nutr. Biochem.* **8:** 112–118.
47. Hertervig, E. 2000. Alkaline Sphingomyelinase, a Potential Inhibitor in Colorectal Carcinogenesis. PhD Dissertation. University of Lund, Lund, Sweden.
48. Nyberg, L., R. Duan, and A. Nilsson. 2000. A mutual inhibitory effect on absorption of sphingomyelin and cholesterol. *J. Nutr. Biochem.* **11:** 244–249.
49. Liu, J. J., A. Nilsson, and R. D. Duan. 2002. In vitro effects of fat, FA, and cholesterol on sphingomyelin hydrolysis induced by rat intestinal alkaline sphingomyelinase. *Lipids.* **37:** 469–474.
50. Duan, R. D., Y. Cheng, H. D. Tauschel, and A. Nilsson. 1998. Effects of ursodeoxycholate and other bile salts on levels of rat intestinal alkaline sphingomyelinase: a potential implication in tumorigenesis. *Dig. Dis. Sci.* **43:** 26–32.
51. Cheng, Y., H. D. Tauschel, A. Nilsson, and R. D. Duan. 1999. Ursodeoxycholic acid increases the activities of alkaline sphingomyelinase and caspase-3 in the rat colon. *Scand. J. Gastroenterol.* **34:** 915–920.
52. Cheng, Y., L. Ohlsson, and R. D. Duan. 2004. Psyllium and fat in diets differentially affect the activities and expressions of colonic sphingomyelinases and caspase in mice. *Br. J. Nutr.* **91:** 715–723.
53. Lillienau, J., Y. Cheng, A. Nilsson, and R. D. Duan. 2003. Development of intestinal alkaline sphingomyelinase in rat fetus and newborn rat. *Lipids.* **38:** 545–549.
54. Nyberg, L. 1998. Digestion and Absorption of Sphingomyelin from Milk. PhD Dissertation. Lund University, Lund, Sweden.
55. Bettger, W. J., E. DiMichelle-Ranalli, B. Dillingham, and C. B. Blackadar. 2003. Nervonic acid is transferred from the maternal diet to milk and tissues of suckling rat pups. *J. Nutr. Biochem.* **14:** 160–165.
56. Schwarz, S. M., B. Hostetler, S. Ling, M. Mone, and J. B. Watkins. 1985. Intestinal membrane lipid composition and fluidity during development in the rat. *Am. J. Physiol.* **248:** G200–G207.
57. Schwarz, S. M., H. E. Bostwick, M. D. Danziger, L. J. Newman, and M. S. Medow. 1989. Ontogeny of basolateral membrane lipid composition and fluidity in small intestine. *Am. J. Physiol.* **257:** G138–G144.
58. Borgström, B. 1980. Importance of phospholipids, pancreatic phospholipase A2, and fatty acid for the digestion of dietary fat: in vitro experiments with the porcine enzymes. *Gastroenterology.* **78:** 954–962.
59. Patton, J. S., and M. C. Carey. 1981. Inhibition of human pancreatic lipase-colipase activity by mixed bile salt-phospholipid micelles. *Am. J. Physiol.* **241:** G328–G336.
60. Moschetta, A., P. M. Frederik, P. Portincasa, G. P. vanBerge-Henegouwen, and K. J. van Erpecum. 2002. Incorporation of cholesterol in sphingomyelin- phosphatidylcholine vesicles has profound effects on detergent-induced phase transitions. *J. Lipid Res.* **43:** 1046–1053.
61. Eckhardt, E. R., D. Q. Wang, J. M. Donovan, and M. C. Carey. 2002. Dietary sphingomyelin suppresses intestinal cholesterol

absorption by decreasing thermodynamic activity of cholesterol monomers. *Gastroenterology.* **122:** 948–956.
62. Noh, S. K., and S. I. Koo. 2003. Egg sphingomyelin lowers the lymphatic absorption of cholesterol and alpha-tocopherol in rats. *J. Nutr.* **133:** 3571–3576.
63. Noh, S. K., and S. I. Koo. 2004. Milk sphingomyelin is more effective than egg sphingomyelin in inhibiting intestinal absorption of cholesterol and fat in rats. *J. Nutr.* **134:** 2611–2616.
64. Chen, H., E. Born, S. N. Mathur, F. C. Johlin, Jr., 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.
65. 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.
66. Garmy, N., N. Taieb, N. Yahi, and J. Fantini. 2005. Interaction of cholesterol with sphingosine: physicochemical characterization and impact on intestinal absorption. *J. Lipid Res.* **46:** 36–45.
67. Motouri, M., H. Matsuyama, J. Yamamura, M. Tanaka, S. Aoe, T. Iwanaga, and H. Kawakami. 2003. Milk sphingomyelin accelerates enzymatic and morphological maturation of the intestine in artificially reared rats. *J. Pediatr. Gastroenterol. Nutr.* **36:** 241–247.
68. Schmelz, E. M. 2004. Sphingolipids in the chemoprevention of colon cancer. *Front. Biosci.* **9:** 2632–2639.
69. Duan, R. D. 2005. Anticancer compounds and sphingolipid metabolism in the colon. *In Vivo.* **19:** 293–300.
70. Schmelz, E. M., P. C. Roberts, E. M. Kustin, L. A. Lemonnier, M. C. Sullards, D. L. Dillehay, and A. H. Merrill, Jr. 2001. Modulation of intracellular beta-catenin localization and intestinal tumorigenesis in vivo and in vitro by sphingolipids. *Cancer Res.* **61:** 6723–6729.
71. Duan, R. D., and A. Nilsson. 2000. Sphingolipid hydrolyzing enzymes in the gastrointestinal tract. *Methods Enzymol.* **311:** 276–286.
72. Hertervig, E., A. Nilsson, L. Nyberg, and R. D. Duan. 1997. Alkaline sphingomyelinase activity is decreased in human colorectal carcinoma. *Cancer.* **79:** 448–453.
73. Hertervig, E., A. Nilsson, J. Bjork, R. Hultkrantz, and R. D. Duan. 1999. Familial adenomatous polyposis is associated with a marked decrease in alkaline sphingomyelinase activity: a key factor to the unrestrained cell proliferation? *Br. J. Cancer.* **81:** 232–236.
74. Di Marzio, L., A. Di Leo, B. Cinque, D. Fanini, A. Agnifili, P. Berloco, M. Linsalata, D. Lorusso, M. Barone, C. De Simone, et al. 2005. Detection of alkaline sphingomyelinase activity in human stool: proposed role as a new diagnostic and prognostic marker of colorectal cancer. *Cancer Epidemiol. Biomarkers Prev.* **14:** 856–862.
75. Hertervig, E., A. Nilsson, Y. Cheng, and R. D. Duan. 2003. Purified intestinal alkaline sphingomyelinase inhibits proliferation without inducing apoptosis in HT-29 colon carcinoma cells. *J. Cancer Res. Clin. Oncol.* **129:** 577–582.
76. Wu, J., Y. Cheng, A. Nilsson, and R. D. Duan. 2004. Identification of one exon deletion of intestinal alkaline sphingomyelinase in colon cancer HT-29 cells and a differentiation-related expression of the wild-type enzyme in Caco-2 cells. *Carcinogenesis.* **25:** 1327–1333.
77. Elferink, R. P., and A. K. Groen. 1999. The mechanism of biliary lipid secretion and its defects. *Gastroenterol. Clin. North Am.* **28:** 59–74, vi.
78. Moschetta, A., G. P. vanBerge-Henegouwen, P. Portincasa, G. Palasciano, A. K. Groen, and K. J. van Erpecum. 2000. Sphingomyelin exhibits greatly enhanced protection compared with egg yolk phosphatidylcholine against detergent bile salts. *J. Lipid Res.* **41:** 916–924.
79. Moschetta, A., P. Portincasa, K. J. van Erpecum, L. Debellis, G. P. Vanberge-Henegouwen, and G. Palasciano. 2003. Sphingomyelin protects against apoptosis and hyperproliferation induced by deoxycholate: potential implications for colon cancer. *Dig. Dis. Sci.* **48:** 1094–1101.
80. Nelson, G. 1967. Phospholipid composition of plasma in various mammalian species. *Lipids.* **2:** 323–329.
81. Dougherty, R. M., C. Galli, A. Ferro-Luzzi, and J. M. Iacono. 1987. Lipid and phospholipid fatty acid composition of plasma, red blood cells, and platelets and how they are affected by dietary lipids: a study of normal subjects from Italy, Finland, and the USA. *Am. J. Clin. Nutr.* **45:** 443–455.
82. Kuksis, A., J. J. Myher, K. Geher, N. A. Shaikh, W. C. Breckenridge, G. J. Jones, and J. A. Little. 1980. Comparative determination of plasma phospholipids by automated gas-liquid chromatographic and manual colorimetric phosphorus methods. *J. Chromatogr.* **182:** 1–26.
83. Bagdade, J. D., and P. V. Subbaiah. 1989. Abnormal high-density lipoprotein composition in women with insulin-dependent diabetes. *J. Lab. Clin. Med.* **113:** 235–240.
84. McIntosh, T. J., S. A. Simon, D. Needham, and C. H. Huang. 1992. Structure and cohesive properties of sphingomyelin/cholesterol bilayers. *Biochemistry.* **31:** 2012–2020.
85. McIntosh, T. J., S. A. Simon, D. Needham, and C. H. Huang. 1992. Interbilayer interactions between sphingomyelin and sphingomyelin/cholesterol bilayers. *Biochemistry.* **31:** 2020–2024.
86. Kan, C. C., R. Bittman, and J. Hajdu. 1991. Phospholipids containing nitrogen- and sulfur-linked chains: kinetics of cholesterol exchange between vesicles. *Biochim. Biophys. Acta.* **1066:** 95–101.
87. Lund-Katz, S., and M. C. Phillips. 1986. Packing of cholesterol molecules in human low-density lipoprotein. *Biochemistry.* **25:** 1562–1568.
88. Murphy, H. C., S. P. Burns, J. J. White, J. D. Bell, and R. A. Iles. 2000. Investigation of human low-density lipoprotein by (1)H nuclear magnetic resonance spectroscopy: mobility of phosphatidylcholine and sphingomyelin headgroups characterizes the surface layer. *Biochemistry.* **39:** 9763–9770.
89. Oorni, K., J. K. Hakala, A. Annila, M. Ala-Korpela, and P. T. Kovanen. 1998. Sphingomyelinase induces aggregation and fusion, but phospholipase A2 only aggregation, of low density lipoprotein (LDL) particles. Two distinct mechanisms leading to increased binding strength of LDL to human aortic proteoglycans. *J. Biol. Chem.* **273:** 29127–29134.
90. Oorni, K., P. Posio, M. Ala-Korpela, M. Jauhiainen, and P. T. Kovanen. 2005. Sphingomyelinase induces aggregation and fusion of small very low-density lipoprotein and intermediate-density lipoprotein particles and increases their retention to human arterial proteoglycans. *Arterioscler. Thromb. Vasc. Biol.* **25:** 1678–1683.
91. Lottin, H., C. Motta, and G. Simard. 1996. Differential effects of glycero- and sphingo-phospholipolysis on human high-density lipoprotein fluidity. *Biochim. Biophys. Acta.* **1301:** 127–132.
92. Rye, K. A., N. J. Hime, and P. J. Barter. 1996. The influence of sphingomyelin on the structure and function of reconstituted high density lipoproteins. *J. Biol. Chem.* **271:** 4243–4250.
93. Green, P. H., and R. M. Glickman. 1981. Intestinal lipoprotein metabolism. *J. Lipid Res.* **22:** 1153–1173.
94. Hamilton, R. L., and P. E. Fielding. 1989. Nascent very low density lipoproteins from rat hepatocytic Golgi fractions are enriched in phosphatidylethanolamine. *Biochem. Biophys. Res. Commun.* **160:** 162–173.
95. Carpentier, A., B. W. Patterson, N. Leung, and G. F. Lewis. 2002. Sensitivity to acute insulin-mediated suppression of plasma free fatty acids is not a determinant of fasting VLDL triglyceride secretion in healthy humans. *Diabetes.* **51:** 1867–1875.
96. Adiels, M., C. Packard, M. J. Caslake, P. Stewart, A. Soro, J. Westerbacka, B. Wennberg, S. O. Olofsson, M. R. Taskinen, and J. Boren. 2005. A new combined multicompartmental model for apolipoprotein B-100 and triglyceride metabolism in VLDL subfractions. *J. Lipid Res.* **46:** 58–67.
97. Marsh, J. B., F. K. Welty, A. H. Lichtenstein, S. Lamon-Fava, and E. J. Schaefer. 2002. Apolipoprotein B metabolism in humans: studies with stable isotope-labeled amino acid precursors. *Atherosclerosis.* **162:** 227–244.
98. Winkler, K. E., and J. B. Marsh. 1989. Characterization of nascent high density lipoprotein subfractions from perfusates of rat liver. *J. Lipid Res.* **30:** 979–987.
99. Brewer, H. B., Jr., A. T. Remaley, E. B. Neufeld, F. Basso, and C. Joyce. 2004. Regulation of plasma high-density lipoprotein levels by the ABCA1 transporter and the emerging role of high-density lipoprotein in the treatment of cardiovascular disease. *Arterioscler. Thromb. Vasc. Biol.* **24:** 1755–1760.
100. Merrill, A. H., Jr., S. Lingrell, E. Wang, M. Nikolova-Karakashian, T. R. Vales, and D. E. Vance. 1995. Sphingolipid biosynthesis de novo by rat hepatocytes in culture. Ceramide and sphingomyelin are associated with, but not required for, very low density lipoprotein secretion. *J. Biol. Chem.* **270:** 13834–13841.
101. Gibbons, G. F., D. Wiggins, A. M. Brown, and A. M. Hebbachi. 2004. Synthesis and function of hepatic very-low-density lipoprotein. *Biochem. Soc. Trans.* **32:** 59–64.
102. Huitema, K., J. van den Dikkenberg, J. F. Brouwers, and J. C. Holthuis. 2004. Identification of a family of animal sphingomyelin synthases. *EMBO J.* **23:** 33–44.
103. Hanada, K., K. Kumagai, S. Yasuda, Y. Miura, M. Kawano, M.

Fukasawa, and M. Nishijima. 2003. Molecular machinery for non-vesicular trafficking of ceramide. *Nature.* **426:** 803–809.
104. Perry, R. J., and N. D. Ridgway. 2005. Molecular mechanisms and regulation of ceramide transport. *Biochim. Biophys. Acta.* **1734:** 220–234.
105. Bearnot, H. R., R. M. Glickman, L. Weinberg, P. H. Green, and A. R. Tall. 1982. Effect of biliary diversion on rat mesenteric lymph apolipoprotein-I and high density lipoprotein. *J. Clin. Invest.* **69:** 210–217.
106. Assmann, G., A. von Eckardstein, and H. B. Brewer, Jr. 2001. Familial high density lipoprotein deficiency: Tangier disease. *In* The Metabolic Basis of Inherited Disease. C. Scriver, A. Beauder, W. Sly, B. Childs, K. Kinzler, and B. Vogelstein, editors. McGraw-Hill, New York. 2937–2980.
107. McNeish, J., R. J. Aiello, D. Guyot, T. Turi, C. Gabel, C. Aldinger, K. L. Hoppe, M. L. Roach, L. J. Royer, J. de Wet, et al. 2000. High density lipoprotein deficiency and foam cell accumulation in mice with targeted disruption of ATP-binding cassette transporter-1. *Proc. Natl. Acad. Sci. USA.* **97:** 4245–4250.
108. Orso, E., C. Broccardo, W. E. Kaminski, A. Bottcher, G. Liebisch, W. Drobnik, A. Gotz, O. Chambenoit, W. Diederich, T. Langmann, et al. 2000. Transport of lipids from Golgi to plasma membrane is defective in Tangier disease patients and Abc1-deficient mice. *Nat. Genet.* **24:** 192–196.
109. Ragozin, S., A. Niemeier, A. Laatsch, B. Loeffler, M. Merkel, U. Beisiegel, and J. Heeren. 2005. Knockdown of hepatic ABCA1 by RNA interference decreases plasma HDL cholesterol levels and influences postprandial lipemia in mice. *Arterioscler. Thromb. Vasc. Biol.* **25:** 1433–1438.
110. Redgrave, T. G. 1971. Association of Golgi membranes with lipid droplets (pre-chylomicrons) from within intestinal epithelial cells during absorption of fat. *Aust. J. Exp. Biol. Med. Sci.* **49:** 209–224.
111. Fernando-Warnakulasuriya, G. J., M. L. Eckerson, W. A. Clark, and M. A. Wells. 1983. Lipoprotein metabolism in the suckling rat: characterization of plasma and lymphatic lipoproteins. *J. Lipid Res.* **24:** 1626–1638.
112. Myher, J. J., A. Kuksis, W. C. Breckenridge, and J. A. Little. 1981. Differential distribution of sphingomyelins among plasma lipoprotein classes. *Can. J. Biochem.* **59:** 626–636.
113. Myher, J. J., A. Kuksis, J. Shepherd, C. J. Packard, J. D. Morrisett, O. D. Taunton, and A. M. Gotto. 1981. Effect of saturated and unsaturated fat diets on molecular species of phosphatidylcholine and sphingomyelin of human plasma lipoproteins. *Biochim. Biophys. Acta.* **666:** 110–119.
114. Reichl, D., and J. M. Sterchi. 1992. Human peripheral lymph lipoproteins are enriched in sphingomyelin. *Biochim. Biophys. Acta.* **1127:** 28–32.
115. Sloop, C. H., L. Dory, and P. S. Roheim. 1987. Interstitial fluid lipoproteins. *J. Lipid Res.* **28:** 225–237.
116. Nanjee, M. N., C. J. Cooke, W. L. Olszewski, and N. E. Miller. 2000. Lipid and apolipoprotein concentrations in prenodal leg lymph of fasted humans. Associations with plasma concentrations in normal subjects, lipoprotein lipase deficiency, and LCAT deficiency. *J. Lipid Res.* **41:** 1317–1327.
117. Nanjee, M. N., C. J. Cooke, R. Garvin, F. Semeria, G. Lewis, W. L. Olszewski, and N. E. Miller. 2001. Intravenous apoA-I/lecithin discs increase pre-beta-HDL concentration in tissue fluid and stimulate reverse cholesterol transport in humans. *J. Lipid Res.* **42:** 1586–1593.
118. Reed, C. F. 1968. Phospholipid exchange between plasma and erythrocytes in man and the dog. *J. Clin. Invest.* **47:** 749–760.
119. Fielding, C. J., and P. E. Fielding. 2001. Cellular cholesterol efflux. *Biochim. Biophys. Acta.* **1533:** 175–189.
120. Kulschar, R., B. Engelmann, C. Brautigam, J. Duhm, J. Thiery, and W. O. Richter. 1995. Fast transmission of alterations in plasma phosphatidylcholine/sphingomyelin ratio and lyso phosphatidylcholine levels into changes of red blood cell membrane phospholipid composition after low density lipoprotein apheresis. *Eur. J. Clin. Invest.* **25:** 258–265.
121. Younsi, M., D. Quilliot, N. Al-Makdissy, I. Delbachian, P. Drouin, M. Donner, and O. Ziegler. 2002. Erythrocyte membrane phospholipid composition is related to hyperinsulinemia in obese non-diabetic women: effects of weight loss. *Metabolism.* **51:** 1261–1268.
122. Zeghari, N., H. Vidal, M. Younsi, O. Ziegler, P. Drouin, and M. Donner. 2000. Adipocyte membrane phospholipids and PPAR-gamma expression in obese women: relationship to hyperinsulinemia. *Am. J. Physiol. Endocrinol. Metab.* **279:** E736–E743.
123. Forte, T. M., R. Goth-Goldstein, R. W. Nordhausen, and M. R. McCall. 1993. Apolipoprotein A-I-cell membrane interaction: extracellular assembly of heterogeneous nascent HDL particles. *J. Lipid Res.* **34:** 317–324.
124. Yancey, P. G., J. K. Bielicki, W. J. Johnson, S. Lund-Katz, M. N. Palgunachari, G. M. Anantharamaiah, J. P. Segrest, M. C. Phillips, and G. H. Rothblat. 1995. Efflux of cellular cholesterol and phospholipid to lipid-free apolipoproteins and class A amphipathic peptides. *Biochemistry.* **34:** 7955–7965.
125. Yokoyama, S. 2005. Assembly of high density lipoprotein by the ABCA1/apolipoprotein pathway. *Curr. Opin. Lipidol.* **16:** 269–279.
126. Knight, B. L. 2004. ATP-binding cassette transporter A1: regulation of cholesterol efflux. *Biochem. Soc. Trans.* **32:** 124–127.
127. Brewer, H. B., Jr., and S. Santamarina-Fojo. 2003. Clinical significance of high-density lipoproteins and the development of atherosclerosis: focus on the role of the adenosine triphosphate-binding cassette protein A1 transporter. *Am. J. Cardiol.* **92:** 10K–16K.
128. Fielding, P. E., K. Nagao, H. Hakamata, G. Chimini, and C. J. Fielding. 2000. A two-step mechanism for free cholesterol and phospholipid efflux from human vascular cells to apolipoprotein A-1. *Biochemistry.* **39:** 14113–14120.
129. Rye, K. A., and P. J. Barter. 2004. Formation and metabolism of prebeta-migrating, lipid-poor apolipoprotein A-I. *Arterioscler. Thromb. Vasc. Biol.* **24:** 421–428.
130. Krimbou, L., M. Tremblay, J. Davignon, and J. S. Cohn. 1998. Association of apolipoprotein E with alpha2-macroglobulin in human plasma. *J. Lipid Res.* **39:** 2373–2386.
131. Huang, Y., A. von Eckardstein, S. Wu, N. Maeda, and G. Assmann. 1994. A plasma lipoprotein containing only apolipoprotein E and with gamma mobility on electrophoresis releases cholesterol from cells. *Proc. Natl. Acad. Sci. USA.* **91:** 1834–1838.
132. Liu, L., A. E. Bortnick, M. Nickel, P. Dhanasekaran, P. V. Subbaiah, S. Lund-Katz, G. H. Rothblat, and M. C. Phillips. 2003. Effects of apolipoprotein A-I on ATP-binding cassette transporter A1-mediated efflux of macrophage phospholipid and cholesterol: formation of nascent high density lipoprotein particles. *J. Biol. Chem.* **278:** 42976–42984.
133. Bielicki, J. K., M. R. McCall, and T. M. Forte. 1999. Apolipoprotein A-I promotes cholesterol release and apolipoprotein E recruitment from THP-1 macrophage-like foam cells. *J. Lipid Res.* **40:** 85–92.
134. Gillotte-Taylor, K., M. Nickel, W. J. Johnson, O. L. Francone, P. Holvoet, S. Lund-Katz, G. H. Rothblat, and M. C. Phillips. 2002. Effects of enrichment of fibroblasts with unesterified cholesterol on the efflux of cellular lipids to apolipoprotein A-I. *J. Biol. Chem.* **277:** 11811–11820.
135. Neufeld, E. B., J. A. Stonik, S. J. Demosky, Jr., C. L. Knapper, C. A. Combs, A. Cooney, M. Comly, N. Dwyer, J. Blanchette-Mackie, A. T. Remaley, et al. 2004. The ABCA1 transporter modulates late endocytic trafficking: insights from the correction of the genetic defect in Tangier disease. *J. Biol. Chem.* **279:** 15571–15578.
136. Ginsberg, H., G. A. Grabowski, J. C. Gibson, R. Fagerstrom, J. Goldblatt, H. S. Gilbert, and R. J. Desnick. 1984. Reduced plasma concentrations of total, low density lipoprotein and high density lipoprotein cholesterol in patients with Gaucher type I disease. *Clin. Genet.* **26:** 109–116.
137. Choi, H. Y., B. Karten, T. Chan, J. E. Vance, W. L. Greer, R. A. Heidenreich, W. S. Garver, and G. A. Francis. 2003. Impaired ABCA1-dependent lipid efflux and hypoalphalipoproteinemia in human Niemann-Pick type C disease. *J. Biol. Chem.* **278:** 32569–32577.
138. Leventhal, A. R., W. Chen, A. R. Tall, and I. Tabas. 2001. Acid sphingomyelinase-deficient macrophages have defective cholesterol trafficking and efflux. *J. Biol. Chem.* **276:** 44976–44983.
139. Mendez, A. J., G. Lin, D. P. Wade, R. M. Lawn, and J. F. Oram. 2001. Membrane lipid domains distinct from cholesterol/sphingomyelin-rich rafts are involved in the ABCA1-mediated lipid secretory pathway. *J. Biol. Chem.* **276:** 3158–3166.
140. Wang, N., D. Lan, M. Gerbod-Giannone, P. Linsel-Nitschke, A. W. Jehle, W. Chen, L. O. Martinez, and A. R. Tall. 2003. ATP-binding cassette transporter A7 (ABCA7) binds apolipoprotein A-I and mediates cellular phospholipid but not cholesterol efflux. *J. Biol. Chem.* **278:** 42906–42912.
141. Bagdade, J. D., W. E. Buchanan, T. Kuusi, and M. R. Taskinen. 1990. Persistent abnormalities in lipoprotein composition in noninsulin-dependent diabetes after intensive insulin therapy. *Arteriosclerosis.* **10:** 232–239.

142. Jadhav, A. V., and G. R. Thompson. 1979. Reversible abnormalities of low density lipoprotein composition in familial hypercholesterolaemia. *Eur. J. Clin. Invest.* **9:** 63–67.
143. Kuksis, A., J. J. Myher, K. Geher, W. C. Breckenridge, and J. A. Little. 1982. Lipid class and molecular species interrelationships among plasma lipoproteins of type III and type IV hyperlipemic subjects. *J. Chromatogr.* **230:** 231–252.
144. Noel, C., Y. L. Marcel, and J. Davignon. 1972. Plasma phospholipids in the different types of primary hyperlipoproteinemia. *J. Lab. Clin. Med.* **79:** 611–621.
145. Chao, F. F., E. J. Blanchette-Mackie, B. F. Dickens, W. Gamble, and H. S. Kruth. 1994. Development of unesterified cholesterol-rich lipid particles in atherosclerotic lesions of WHHL and cholesterol-fed NZW rabbits. *J. Lipid Res.* **35:** 71–83.
146. Daugherty, A., B. S. Zweifel, B. E. Sobel, and G. Schonfeld. 1988. Isolation of low density lipoprotein from atherosclerotic vascular tissue of Watanabe heritable hyperlipidemic rabbits. *Arteriosclerosis.* **8:** 768–777.
147. Hara, A., and T. Taketomi. 1990. Characterization and change of phospholipids in the aorta of Watanabe hereditable hyperlipidemic rabbit. *Jpn. J. Exp. Med.* **60:** 311–318.
148. de Roos, B., M. J. Caslake, K. Milliner, G. M. Benson, K. E. Suckling, and C. J. Packard. 2005. Characterisation of the lipoprotein structure in the St. Thomas' Mixed Hyperlipidaemic (SMHL) rabbit. *Atherosclerosis.* **181:** 63–68.
149. Merrill, A. H., Jr., E. Wang, W. S. Innis, and R. Mullins. 1985. Increases in serum sphingomyelin by 17 beta-estradiol. *Lipids.* **20:** 252–254.
150. Walzem, R. L., R. J. Hansen, D. L. Williams, and R. L. Hamilton. 1999. Estrogen induction of VLDLy assembly in egg-laying hens. *J. Nutr.* **129 (Suppl.):** 467–472.
151. Herrera, E., and E. Amusquivar. 2000. Lipid metabolism in the fetus and the newborn. *Diabetes Metab. Res. Rev.* **16:** 202–210.
152. Schlitt, A., M. R. Hojjati, H. von Gizycki, K. J. Lackner, S. Blankenberg, B. Schwaab, J. Meyer, H. J. Rupprecht, and X. C. Jiang. 2005. Serum sphingomyelin levels are related to the clearance of postprandial remnant-like particles. *J. Lipid Res.* **46:** 196–200.
153. Verseyden, C., S. Meijssen, and M. Castro Cabezas. 2002. Postprandial changes of apoB-100 and apoB-48 in TG rich lipoproteins in familial combined hyperlipidemia. *J. Lipid Res.* **43:** 274–280.
154. Martins, I. J., and T. G. Redgrave. 2004. Obesity and post-prandial lipid metabolism. Feast or famine? *J. Nutr. Biochem.* **15:** 130–141.
155. Xiang, S. Q., K. Cianflone, D. Kalant, and A. D. Sniderman. 1999. Differential binding of triglyceride-rich lipoproteins to lipoprotein lipase. *J. Lipid Res.* **40:** 1655–1663.
156. Tall, A. R., P. H. Green, R. M. Glickman, and J. W. Riley. 1979. Metabolic fate of chylomicron phospholipids and apoproteins in the rat. *J. Clin. Invest.* **64:** 977–989.
157. Redgrave, T. G., and D. M. Small. 1979. Quantitation of the transfer of surface phospholipid of chylomicrons to the high density lipoprotein fraction during the catabolism of chylomicrons in the rat. *J. Clin. Invest.* **64:** 162–171.
158. Tam, S. P., and W. C. Breckenridge. 1983. Apolipoprotein and lipid distribution between vesicles and HDL-like particles formed during lipolysis of human very low density lipoproteins by perfused rat heart. *J. Lipid Res.* **24:** 1343–1357.
159. Jiang, X. C., C. Bruce, J. Mar, M. Lin, Y. Ji, O. L. Francone, and A. R. Tall. 1999. Targeted mutation of plasma phospholipid transfer protein gene markedly reduces high-density lipoprotein levels. *J. Clin. Invest.* **103:** 907–914.
160. Qin, S., K. Kawano, C. Bruce, M. Lin, C. Bisgaier, A. R. Tall, and X. Jiang. 2000. Phospholipid transfer protein gene knock-out mice have low high density lipoprotein levels, due to hypercatabolism, and accumulate apoA-IV-rich lamellar lipoproteins. *J. Lipid Res.* **41:** 269–276.
161. Landin, B., A. Nilsson, J. S. Twu, and M. C. Schotz. 1984. A role for hepatic lipase in chylomicron and high density lipoprotein phospholipid metabolism. *J. Lipid Res.* **25:** 559–563.
162. Scow, R. O., and T. Egelrud. 1976. Hydrolysis of chylomicron phosphatidylcholine in vitro by lipoprotein lipase, phospholipase A2 and phospholipase C. *Biochim. Biophys. Acta.* **431:** 538–549.
163. Minari, O., and D. B. Zilversmit. 1963. Behavior of dog lymph chylomicron lipid constituents during incubation with serum. *J. Lipid Res.* **4:** 424–436.
164. Wood, P., K. Imaichi, J. Knowles, G. Michaels, and L. Kinsell. 1964. The lipid composition of human plasma chylomicrons. *J. Lipid Res.* **5:** 225–231.
165. Agren, J. J., J. P. Kurvinen, and A. Kuksis. 2005. Isolation of very low density lipoprotein phospholipids enriched in ethanolamine phospholipids from rats injected with Triton WR 1339. *Biochim. Biophys. Acta.* **1734:** 34–43.
166. Rao, R., J. J. Albers, G. Wolfbauer, and H. J. Pownall. 1997. Molecular and macromolecular specificity of human plasma phospholipid transfer protein. *Biochemistry.* **36:** 3645–3653.
167. Krimbou, L., M. Tremblay, H. Jacques, J. Davignon, and J. S. Cohn. 1998. In vitro factors affecting the concentration of gamma-LpE (γ-LpE) in human plasma. *J. Lipid Res.* **39:** 861–872.
168. Redgrave, T. G. 2004. Chylomicron metabolism. *Biochem. Soc. Trans.* **32:** 79–82.
169. Packard, C. J., and J. Shepherd. 1997. Lipoprotein heterogeneity and apolipoprotein B metabolism. *Arterioscler. Thromb. Vasc. Biol.* **17:** 3542–3556.
170. Illingworth, D. R., and O. W. Portman. 1972. Exchange of phospholipids between low and high density lipoproteins of squirrel monkeys. *J. Lipid Res.* **13:** 220–227.
171. Marques-Vidal, P., M. Jauhiainen, J. Metso, and C. Ehnholm. 1997. Transformation of high density lipoprotein 2 particles by hepatic lipase and phospholipid transfer protein. *Atherosclerosis.* **133:** 87–95.
172. Zilversmit, D. B., C. Entenman, and I. Chaikoff. 1948. The measurement of turnover of the various phospholipids in liver and plasma of the dog and its application to the mechanism of action of choline. *J. Biol. Chem.* **176:** 193–208.
173. Zilversmit, D. B., C. Entenman, and I. Chaikoff. 1948. The turnover rates of plasma lecithin and plasma sphingomyelin as measured by the disappearance of their radioactive phosphorus from the circulation. *J. Biol. Chem.* **176:** 209–212.
174. Bentejac, M., J. Lecerf, M. Bugaut, and M. C. Delachambre. 1988. Turnover and uptake of double-labelled high-density lipoprotein sphingomyelin in the adult rat. *Biochim. Biophys. Acta.* **959:** 349–360.
175. Bentejac, M., M. Bugaut, M. C. Delachambre, and J. Lecerf. 1990. Metabolic fate of sphingomyelin of high-density lipoprotein in rat plasma. *Lipids.* **25:** 653–660.
176. Bentejac, M., M. Bugaut, M. C. Delachambre, and J. Lecerf. 1990. Time-course of utilization of [stearic or lignoceric acid]sphingomyelin from high-density lipoprotein by rat tissues. *Biochim. Biophys. Acta.* **1043:** 134–142.
177. Stein, O., K. Oette, D. Haratz, G. Halperin, and Y. Stein. 1988. Sphingomyelin liposomes with defined fatty acids: metabolism and effects on reverse cholesterol transport. *Biochim. Biophys. Acta.* **960:** 322–333.
178. Stein, Y., K. Oette, Y. Dabach, G. Hollander, M. Ben-Naim, and O. Stein. 1991. Metabolism of 3-[^{3}H]sphingosine sphingomyelin labeled with [^{14}C]palmitic or [^{14}C]linoleic acid by Hep G2 cells and rat liver in vivo. *Biochim. Biophys. Acta.* **1084:** 87–93.
179. Thuahnai, S. T., S. Lund-Katz, D. L. Williams, and M. C. Phillips. 2001. Scavenger receptor class B, type I-mediated uptake of various lipids into cells. Influence of the nature of the donor particle interaction with the receptor. *J. Biol. Chem.* **276:** 43801–43808.
180. Urban, S., S. Zieseniss, M. Werder, H. Hauser, R. Budzinski, and B. Engelmann. 2000. Scavenger receptor BI transfers major lipoprotein-associated phospholipids into the cells. *J. Biol. Chem.* **275:** 33409–33415.
181. Webb, N. R., M. C. de Beer, F. C. de Beer, and D. R. van der Westhuyzen. 2004. ApoB-containing lipoproteins in apoE-deficient mice are not metabolized by the class B scavenger receptor BI. *J. Lipid Res.* **45:** 272–280.
182. Gatt, S., and E. L. Bierman. 1980. Sphingomyelin suppresses the binding and utilization of low density lipoproteins by skin fibroblasts. *J. Biol. Chem.* **255:** 3371–3376.
183. Levade, T., S. Gatt, A. Maret, and R. Salvayre. 1991. Different pathways of uptake and degradation of sphingomyelin by lymphoblastoid cells and the potential participation of the neutral sphingomyelinase. *J. Biol. Chem.* **266:** 13519–13529.
184. Verdery, R. B., 3rd, and R. Theolis, Jr. 1982. Regulation of sphingomyelin long chain base synthesis in human fibroblasts in culture. Role of lipoproteins and the low density lipoprotein receptor. *J. Biol. Chem.* **257:** 1412–1417.
185. Kuksis, A., W. C. Breckenridge, J. J. Myher, and G. Kakis. 1978. Replacement of endogenous phospholipids in rat plasma lipoproteins during intravenous infusion of an artificial lipid emulsion. *Can. J. Biochem.* **56:** 630–639.
186. Redgrave, T. G., V. Rakic, B. C. Mortimer, and J. C. Mamo. 1992. Effects of sphingomyelin and phosphatidylcholine acyl chains on

the clearance of triacylglycerol-rich lipoproteins from plasma. Studies with lipid emulsions in rats. *Biochim. Biophys. Acta.* **1126:** 65–72.
187. Saito, H., I. Arimoto, M. Tanaka, T. Sasaki, T. Tanimoto, S. Okada, and T. Handa. 2000. Inhibition of lipoprotein lipase activity by sphingomyelin: role of membrane surface structure. *Biochim. Biophys. Acta.* **1486:** 312–320.
188. Arimoto, I., H. Saito, Y. Kawashima, K. Miyajima, and T. Handa. 1998. Effects of sphingomyelin and cholesterol on lipoprotein lipase-mediated lipolysis in lipid emulsions. *J. Lipid Res.* **39:** 143–151.
189. Lobo, L. I., and D. C. Wilton. 1997. Combined effects of sphingomyelin and cholesterol on the hydrolysis of emulsion particle triolein by lipoprotein lipase. *Biochim. Biophys. Acta.* **1349:** 122–130.
190. Cantin, B., L. D. Brun, C. Gagne, M. R. Murthy, P. J. Lupien, and P. Julien. 1992. Alterations in erythrocyte membrane lipid composition and fluidity in primary lipoprotein lipase deficiency. *Biochim. Biophys. Acta.* **1139:** 25–31.
191. Arimoto, I., C. Matsumoto, M. Tanaka, K. Okuhira, H. Saito, and T. Handa. 1998. Surface composition regulates clearance from plasma and triolein lipolysis of lipid emulsions. *Lipids.* **33:** 773–779.
192. Morita, S. Y., M. Nakano, A. Sakurai, Y. Deharu, A. Vertut-Doi, and T. Handa. 2005. Formation of ceramide-enriched domains in lipid particles enhances the binding of apolipoprotein E. *FEBS Lett.* **579:** 1759–1764.
193. Morita, S. Y., K. Okuhira, N. Tsuchimoto, A. Vertut-Doi, H. Saito, M. Nakano, and T. Handa. 2003. Effects of sphingomyelin on apolipoprotein E- and lipoprotein lipase-mediated cell uptake of lipid particles. *Biochim. Biophys. Acta.* **1631:** 169–176.
194. Krieger, M. 1999. Charting the fate of the "good cholesterol": identification and characterization of the high-density lipoprotein receptor SR-BI. *Annu. Rev. Biochem.* **68:** 523–558.
195. Van Eck, M., M. Pennings, M. Hoekstra, R. Out, and T. J. Van Berkel. 2005. Scavenger receptor BI and ATP-binding cassette transporter A1 in reverse cholesterol transport and atherosclerosis. *Curr. Opin. Lipidol.* **16:** 307–315.
196. Subbaiah, P. V., and M. Liu. 1993. Role of sphingomyelin in the regulation of cholesterol esterification in the plasma lipoproteins. Inhibition of lecithin-cholesterol acyltransferase reaction. *J. Biol. Chem.* **268:** 20156–20163.
197. Bolin, D. J., and A. Jonas. 1996. Sphingomyelin inhibits the lecithin-cholesterol acyltransferase reaction with reconstituted high density lipoproteins by decreasing enzyme binding. *J. Biol. Chem.* **271:** 19152–19158.
198. Lindholm, E. M., J. K. Bielicki, L. K. Curtiss, E. M. Rubin, and T. M. Forte. 1998. Deletion of amino acids Glu146Arg160 in human apolipoprotein A-I (ApoA-ISeattle) alters lecithin:cholesterol acyltransferase activity and recruitment of cell phospholipid. *Biochemistry.* **37:** 4863–4868.
199. Dhansay, M. A., A. J. Benade, and P. R. Donald. 1991. Plasma lecithin-cholesterol acyltransferase activity and plasma lipoprotein composition and concentrations in kwashiorkor. *Am. J. Clin. Nutr.* **53:** 512–519.
200. Kawano, K., S. C. Qin, M. Lin, A. R. Tall, and X. C. Jiang. 2000. Cholesteryl ester transfer protein and phospholipid transfer protein have nonoverlapping functions in vivo. *J. Biol. Chem.* **275:** 29477–29481.
201. Thuahnai, S. T., S. Lund-Katz, P. Dhanasekaran, M. de la Llera-Moya, M. A. Connelly, D. L. Williams, G. H. Rothblat, and M. C. Phillips. 2004. Scavenger receptor class B type I-mediated cholesteryl ester-selective uptake and efflux of unesterified cholesterol. Influence of high density lipoprotein size and structure. *J. Biol. Chem.* **279:** 12448–12455.
202. McCandless, E. L., and D. B. Zilversmit. 1956. The effect of cholesterol on the turnover of lecithin, cephalin and sphingomyelin in the rabbit. *Arch. Biochem. Biophys.* **62:** 402–410.
203. Rodriguez, J., A. Catapano, G. C. Ghiselli, and C. R. Sirtori. 1976. Turnover and aortic uptake of very low density lipoproteins (VLDL) from hypercholesteremic rabbits as a model for testing antiatherosclerotic compounds. *Adv. Exp. Med. Biol.* **67:** 169–189.
204. Geelen, M. J., L. B. Tijburg, C. J. Bouma, and A. C. Beynen. 1995. Cholesterol consumption alters hepatic sphingomyelin metabolism in rats. *J. Nutr.* **125:** 2294–2300.
205. Benade, A. J., J. E. Fincham, C. M. Smuts, M. T. Tung, D. Chalton, M. Kruger, M. J. Weight, A. K. Daubitzer, and H. Y. Tichelaar. 1988. Plasma low density lipoprotein composition in relation to atherosclerosis in nutritionally defined vervet monkeys. *Atherosclerosis.* **74:** 157–168.
206. Kruger, M., C. M. Smuts, A. J. Benade, J. E. Fincham, C. J. Lombard, E. A. Albertse, and K. J. van der Merwe. 1992. Comparison of the effect of the amount and degree of unsaturation of dietary fat on plasma low density lipoproteins in vervet monkeys. *Lipids.* **27:** 733–739.
207. Parks, J. S., and A. K. Gebre. 1991. Studies on the effect of dietary fish oil on the physical and chemical properties of low density lipoproteins in cynomolgus monkeys. *J. Lipid Res.* **32:** 305–315.
208. Geelen, M. J., and A. C. Beynen. 2000. Consumption of olive oil has opposite effects on plasma total cholesterol and sphingomyelin concentrations in rats. *Br. J. Nutr.* **83:** 541–547.
209. Geelen, M. J., D. van Hoorn, and A. C. Beynen. 1999. Consumption of casein instead of soybean protein produces a transient rise in the concentration of sphingomyelin in VLDL in rats. *J. Nutr.* **129:** 2119–2122.
210. Bagdade, J. D., W. E. Buchanan, R. A. Levy, P. V. Subbaiah, and M. C. Ritter. 1990. Effects of omega-3 fish oils on plasma lipids, lipoprotein composition, and postheparin lipoprotein lipase in women with IDDM. *Diabetes.* **39:** 426–431.
211. Fievet, C., O. Ziegler, H. J. Parra, L. Mejean, J. C. Fruchart, and P. Drouin. 1990. Depletion in choline containing phospholipids of LpB particles in adequately controlled type I insulin-dependent diabetes mellitus. *Diabete Metab.* **16:** 64–69.
212. Li, Z., M. J. Basterr, T. K. Hailemariam, M. R. Hojjati, S. Lu, J. Liu, R. Liu, H. Zhou, and X. C. Jiang. 2005. The effect of dietary sphingolipids on plasma sphingomyelin metabolism and atherosclerosis. *Biochim. Biophys. Acta.* **1735:** 130–134.
213. Kobayashi, T., T. Shimizugawa, T. Osakabe, S. Watanabe, and H. Okuyama. 1997. A long-term feeding of sphingolipids affected the levels of plasma-cholesterol and hepatic triacylglycerol but not tissue phospholipids and sphingolipids. *Nutr. Res.* **17:** 111–114.
214. Imaizumi, K., A. Tomaga, M. Sato, and M. Sugano. 1992. Effects of dietary sphingolipids on levels of serum and liver lipids in rats. *Nutr. Res.* **12:** 543–548.
215. Bladergroen, B. A., A. C. Beynen, and M. J. Geelen. 1999. Dietary pectin lowers sphingomyelin concentration in VLDL and raises hepatic sphingomyelinase activity in rats. *J. Nutr.* **129:** 628–633.
216. Piperi, C., C. Kalofoutis, K. Skenderi, O. Economidou, and A. Kalofoutis. 2004. Beneficial effects of raloxifene and atorvastatin on serum lipids and HDL phospholipids levels of postmenopausal women. *J. Obstet. Gynaecol.* **24:** 414–419.
217. Ozerova, I. N., I. V. Paramonova, A. M. Olfer'ev, N. M. Akhmedzhanov, M. A. Aleksandrova, and N. V. Perova. 2001. Effects of simvastatin on the phospholipid composition of high-density lipoproteins in patients with hypercholesterolemia. *Bull. Exp. Biol. Med.* **132:** 763–765.
218. Park, E. J., M. Suh, K. Ramanujam, K. Steiner, D. Begg, and M. T. Clandinin. 2005. Diet-induced changes in membrane gangliosides in rat intestinal mucosa, plasma and brain. *J. Pediatr. Gastroenterol. Nutr.* **40:** 487–495.
219. Malhotra, S., and D. Kritchevsky. 1978. The distribution and lipid composition of ultracentrifugally separated lipoproteins of young and old rat plasma. *Mech. Ageing Dev.* **8:** 445–452.
220. Gold, M., and H. Altschuler. 1972. Red blood cell and plasma phospholipids in aged humans. *J. Gerontol.* **27:** 444–450.
221. Cutler, R. G., and M. P. Mattson. 2001. Sphingomyelin and ceramide as regulators of development and lifespan. *Mech. Ageing Dev.* **122:** 895–908.
222. Subbaiah, P. V., V. S. Subramanian, and K. Wang. 1999. Novel physiological function of sphingomyelin in plasma. Inhibition of lipid peroxidation in low density lipoproteins. *J. Biol. Chem.* **274:** 36409–36414.