CHAPTER 2

SPHINGOLIPIDS AND CARDIOVASCULAR DISEASES:

Lipoprotein Metabolism, Atherosclerosis and Cardiomyopathy

Xian-Cheng Jiang,*[,1] Ira J. Goldberg[2] and Tae-Sik Park[3]

[1]Department of Cell Biology, Downstate Medical Center, State University of New York, Brooklyn, New York, New York, USA; [2]Department of Medicine, Division of Preventive Medicine and Nutrition, Columbia University, New York, New York, USA; [3]Lee Gil Ya Cancer and Diabetes Institute, Gachon University of Medicine and Science, Inchon, South Korea

**Corresponding Author: Xian-Cheng Jiang—Email: xian-cheng.jiang@downstate.edu*

Abstract: Heart disease is widely believed to develop from two pathological processes. Circulating lipoproteins containing the nondegradable lipid, cholesterol, accumulate within the arterial wall and perhaps are oxidized to more toxic lipids. Both lipid accumulation and vascular reaction to the lipids lead to the gradual thickening of the vascular wall. A second major process that in some circumstances is a primary event is the development of a local inflammatory reaction. This might be a reaction to vessel wall injury that accompanies infections, immune disease, and perhaps diabetes and renal failure. In this chapter, we will focus on the relationship between de novo synthesis of sphingolipids and lipid metabolism, atherosclerosis, and cardiomyopathy.

INTRODUCTION

Coronary artery disease is widely believed to develop from two pathological processes. Circulating lipoproteins containing the nondegradable lipid, cholesterol, accumulate within the arterial wall and perhaps are oxidized to more toxic lipids. Both lipid accumulation and vascular reaction to the lipids lead to the gradual thickening of the vascular wall. A second major process that in some circumstances is a primary event is the development

Sphingolipids and Metabolic Disease, edited by L. Ashley Cowart.

of a local inflammatory reaction. This might be a reaction to vessel wall injury that accompanies infections, immune disease and perhaps diabetes and renal failure.

Lipid accumulation is associated with a number of other diseases. These include nonalcoholic liver disease, Type 2 diabetes due to reduced insulin secretion[1] and skeletal muscle insulin resistance[2] and some forms of cardiomyopathy.[3] Several mechanisms have been proposed to explain how lipid accumulation leads to organ dysfunction: (1) direct toxic effects of neutral droplets or fatty acids (FAs) on myofibrillar function[4] (2) reactive oxygen species (ROS) generated as a toxic by-product of lipid oxidation[4] (3) FA-induced apoptosis[3] (4) diacylglycerol induced activation of signaling pathways such as those mediated by protein kinase Cs.[5] When the balance between oxidation and FA uptake is altered, excess FA must enter pathways for nonoxidative metabolism. This anomalous lipid metabolism is thought to lead to dysfunction of nonadipose tissues. One alternative route to utilize the FA surplus is via the sphingolipid biosynthetic pathway.

Dysregulation of the sphingolipid biosynthetic pathways associated with excess FA uptake and accumulation may be a central metabolic derangement in lipotoxicity and atherosclerosis. This could occur due to changes in plasma levels of circulating lipids or inappropriate lipid uptake by cells/tissues. Correction of the induced sphingolipid biosynthesis excess could become a treatment for diseases such as atherosclerosis and lipotoxicities. Sphingomyelin (SM) is one of the major lipid components in plasma and of cell membranes. Plasma SM levels are an independent risk factor for coronary artery disease, i.e.,independent of cholesterol.[6] In mice, reduction of plasma and liver SM leads to a concomitant reduction of atherosclerosis; this was achieved by pharmacological inhibition of serine palmitoyltransferase (SPT).[7,8] Macrophage deficiency of sphingomyelin synthase 2 (SMS2), the last enzyme for SM biosynthesis, decreases plasma membrane SM levels and decreases atherosclerosis in a mouse model.[9] Cardiac dysfunction caused by lipid accumulation in heart[10] and insulin resistance associated with dietary or infused saturated FAs[11] is ameliorated by inhibition of ceramide biosynthesis. These observations emphasize the need for a better understanding of sphingolipid metabolism in cardiovascular diseases. In this chapter, we will focus on the relationship between de novo synthesis of sphingolipids and lipid metabolism, atherosclerosis and cardiomyopathy.

SPHINGOLIPID BIOSYNTHESIS

The biochemical synthesis of SM occurs through a series of reactions involving the enzymes serine palmitoyltransferase (SPT), 3-ketosphinganine reductase, ceramide synthase, dihydroceramide desaturase and sphingomyelin synthase (Fig. 1).

Located in the endoplasmic reticulum (ER) membrane, SPT is the rate-limiting enzyme in the pathway.[12] Mammalian SPT contains two subunits, Sptlc1 and Sptlc2, encoding 53- and 63-kDa proteins, respectively.[13,14] The subunits are homologous (about 20% identity)[13,14] and this is probably relevant to their formation of a heterodimer. Both Sptlc1 and Sptlc2 have a single, highly hydrophobic N-terminal transmembrane domain.[13,14] Neither appears to be glycosylated.[13] Indirect immunocytochemical analysis with epitope-tagged Sptlc1 indicates that the N- and C-termini of both subunits are oriented to the lumen and cytosol, respectively.[15] A third possible subunit, Sptlc3[16] has 68% homology with Sptlc2 and 20% homology with Sptlc1. Over-expression of Sptlc3 in Hek293 cells led to a 2-3 fold increase in cellular SPT activity.

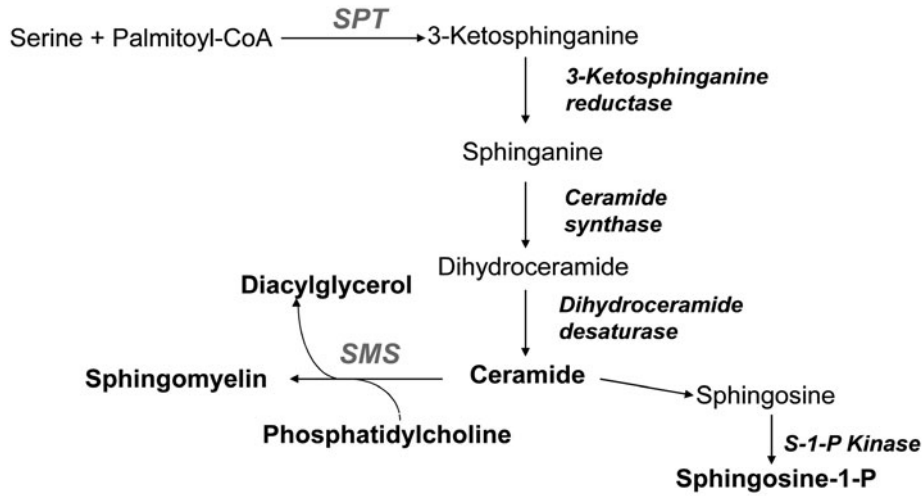

Figure 1. Sphingolipid biosynthesis in mammals. SPT, serine palmitoyltransferase; SMS, sphingomyelin synthase.

Silencing of Sptlc3 expression in HepG2 cells resulted in a significant reduction of cellular SPT activity, but not a complete loss.[16] The authors speculated that Sptlc3 was an isoform of Sptlc2, each binding Sptlc1 independently and that varying the amounts of Sptlc2 and -3 might be a cellular mechanism to adjust SPT activity to meet tissue specific requirements for sphingolipids.

In yeast SPT is composed of a heterodimer of 2 highly-related subunits, Lcb1p and Lcb2p, and a third subunit, Tsc3p, which increases enzyme activity markedly and is required for growth at elevated temperatures.[17] Recently, 2 proteins, ssSPTa and ssSPTb, which despite sharing no homology with Tsc3p, substantially enhances the activity of mammalian SPT expressed in either yeast or mammalian cells, were identified. These proteins are evidence for an evolutionarily conserved family of low molecular weight proteins that confer full SPT enzyme activity.[18] The small subunits of mammalian SPT confer distinct acyl-CoA substrate specificities.[18] SMS is located mainly in the *cis*-, medial-Golgi[19-21] and plasma membranes.[22-24]

There may also be a form of SMS in the *trans*-Golgi network[25] and the nucleus.[26] SMS activity has been found in chromatin and chromatin-associated SMS modifies the SM content.[27] Two SMS genes, SMS1 and SMS2, have been cloned and characterized for their cellular localization.[28,29] SMS1 is found in the *trans*-Golgi apparatus, while SMS2 is predominantly found in the plasma membranes[28,30] and also Golgi apparatus.[28,30] SMS1 and -2 expression positively correlates with levels of cellular SM and SM in lipid rafts.[31-33]

The sphingolipid biosynthesis pathway impacts cellular production of at least three bioactive lipids: ceramide, diglyceride, and sphingosine-1-phosphate; and two structure-related lipids: sphingomyelin and phosphatidylcholine. In this chapter, we focus on sphingomyelin and ceramide.

ROLE OF SPHINGOMYELIN (SM) IN LIPOPROTEIN METABOLISM

Sphingomyelin (SM) is a major component of cell membranes and both atherogenic and anti-atherogenic lipoproteins. Chylomicrons, very low density lipoprotein (VLDL) and low density lipoprotein (LDL) are atherogenic lipoproteins, while high density lipoproteins (HDL) are antiatherogenic ones. Chylomicrons, VLDL and LDL are enriched with SM.[1]

SM and Chylomicron/VLDL Metabolism

The primary metabolic role of VLDL and chylomicrons is to transport endogenous and dietary triglyceride used for energy, structural lipids and storage. Triglyceride located on these particles is hydrolyzed into free FAs by lipoprotein lipase (LpL) associated with the luminal surface of capillaries.[34,35] In vitro studies indicate that SM in these lipoproteins inhibits the action of LpL (Fig. 2). Kuksis et al[36] compared LpL hydrolysis of TG using three emulsions as substrates for the enzyme: phosphatidylcholine (PC)/TG, PC/SM/TG, and PC/phosphatidylethanoloamine (PE)/TG. They found that SM-containing emulsion had the lowest TG clearance rate in vitro. Saito et al[37] and Arimoto et al[38] found that SM, but not cholesterol, significantly inhibited TG lipolysis by LpL in a SM concentration-dependent manner. Lobo and Wilton[39] and Cantin et al[40] showed that cholesterol inclusion in the PC surface coat of a TG emulsion stimulated lipolysis by LpL, but that this stimulation was eliminated by adding SM.

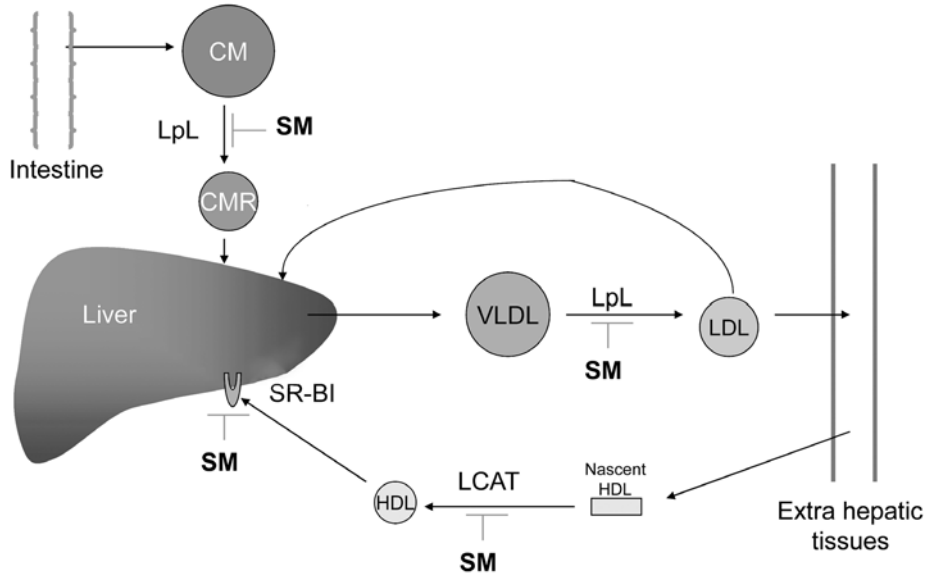

Figure 2. Role of SM in regulation of lipoprotein metabolism. Increased plasma SM inhibits lipoprotein lipase (LpL) and hydrolysis of triglyceride in chylomicrons and VLDL. This latter action reduces the production of LDL. Maturation of nascent HDL is inhibited by SM. SM inhibits LCAT activity and formation of cholesterol ester in HDL is reduced. SM also inhibits SR-BI, a HOL receptor.

SM and Apolipoprotein E-Containing Lipoprotein Metabolism

Apolipoprotein E (ApoE) is a protein which has anti-atherogenic property. Bound to plasma lipoproteins, it is a ligand for cell uptake by the LDL receptor (LDLR), the LDL receptor-related protein (LRP) and heparan sulfate proteoglycans.[41,42] ApoE also influences the assembly and secretion of lipoproteins[43,44] and cholesterol efflux to HDL.[45] Arimoto et al reported that SM in lipoproteins delays remnant clearance by decreasing the binding of apoE to cell membrane receptors.[46] Sphingomyelinase causes particle aggregation in artificial lipid emulsions of TG/PC/SM by degradation of SM to ceramide. ApoE prevents this aggregation,[47] implying an interaction between SM and apoE. To clarify the role of SM in lipoprotein uptake, Morita et al[48] prepared lipid emulsions containing triolein, PC and SM as model particles of reconstituted lipoproteins. Incorporation of SM into the emulsion surface reduced the binding capacity of apoE without changing its affinity for the receptors on the surface of hepatocytes. Surface SM reduced apoE-mediated uptake of emulsions by HepG2 cells because of the decreased amount of binding apoE. The stimulatory effect of LpL on emulsion uptake was decreased by replacing surface PC with SM. These results suggest that SM-induced changes in the binding properties of apoE and LpL correlate with decreased hepatic uptake of lipid particles. Lucic et al found that apoE expression also increased SM secretion from macrophages and this SM was colocalized with apoE in secreted lipoprotein particles.[49] Although the in vivo significance of these findings to apoE-containing lipoprotein metabolism is not yet established, they may be highly relevant during the increase in the SM content of plasma lipoproteins that occurs following a large lipid load[50] or apoE deficiency.[51]

SM and HDL Metabolism

HDLs are well-known anti-atherogenic lipoproteins. HDL particles are involved in the process of reverse cholesterol transport which removes cholesterol from peripheral tissues and cells. The cholesterol on HDL becomes cholesterol ester (CE) through the activity of lecithin:cholesterol acyltransferase (LCAT). The CE of HDL may then be delivered to liver or steroidogenic tissues by the scavenger receptor B1 (SR-B1)[52,53] (primary fate) or transferred, via plasma cholesteryl ester transfer protein (CETP) to LDL and TG-rich lipoproteins in exchange for TG.[54]

These processes may be affected by the presence of SM in HDL. SM inhibits LCAT by decreasing its binding to HDL[55] (Fig. 2). A negative correlation between the SM content of HDL and LCAT activity was observed in studies with proteoliposomes or reconstituted HDL.[56]

Rye, Hime and Barter[57] found that SM influences the structure of discoidal and spherical HDL and confirmed that SM inhibits the LCAT reaction.

Macrophage cholesterol efflux plays an important role in reverse cholesterol transport, an anti-atherogenic process.[58] However the capacity of mouse plasma to mediate cholesterol efflux from mouse macrophages supplemented with different concentrations of SM was not altered. This result indicated that plasma SM levels might not have a direct effect on cholesterol efflux (Li and Jiang unpublished observation).

SR-BI is the first molecularly defined receptor for HDL and can mediate the selective uptake of CE into cells. Subbaiah et al[59] investigated the effect of SM in lipoproteins on the selective uptake in three different cell lines: SR-BI-transfected CHO cells, hepatocytes

(HepG2) and adrenocortical cells (Y1BS1). They found that SM in the lipoproteins regulates the SR-BImediated selective uptake of CE, possibly by interacting with the sterol ring or with SR-BI itself.

A key function of HDL SM may be to regenerate HDL during normal lipid metabolism such as after liver SR-B1 extracts CE from spherical HDL and releases some lipid-free apoA1 protein. Addition of SM-enriched phospholipids and cholesterol to apoA1 produces a new lipoprotein species optimized to accept cholesterol from cells, thus restarting HDL growth.[60]

SPHINGOMYELIN AND ATHEROSCLEROSIS

Atherosclerosis is an inflammatory disease characterized by the production of a wide range of chemokines and cytokines. Atherogenesis is initiated by the interaction of cholesterol-rich lipoproteins with the arterial wall.[61] Many processes have been implicated in early atherogenesis, including lipoprotein oxidation,[62,63] lipoprotein retention and aggregation,[64-67] endothelial alteration,[61] monocyte recruitment, macrophage chemotaxis and foam cell formation[61] and smooth muscle cell migration and alteration.[61]

Two sets of evidence indicate that both the amount of SM in the aortic wall and the SM levels in plasma are closely related to the development of atherosclerosis: (1) SM accumulates in atheromas formed in human and animal models.[68-73] LDL extracted from human atherosclerotic lesions is much richer in SM than LDL from plasma.[74-77] A substantial amount of the SM found in arteries and atherosclerotic lesions appears to arise from synthesis in the arterial tissues.[78,79] SM concentration is also significantly increased in macrophages treated with acetyl-LDL plus an acyl-CoA:cholesterol acyltransferase (ACAT) inhibitor.[80] (2) Even in atherosclerotic lesions, the rate of SM formation is relatively slow compared with the rate of total choline-containing phospholipid synthesis,[81] suggesting that additional factors contribute to intimal SM accumulation. The ratio of SM to PC is increased by 5-fold in VLDL from hypercholesterolemic rabbits.[82] ApoE knockout (KO) mice are a well known atherogenic model. Plasma SM levels in these mice are 4-fold higher than in wild type mice[51] and this may contribute to the increased atherosclerosis.[83,84] We also found that human plasma SM levels and SM/PC ratio are independent risk factors for coronary heart disease.[6,85] Moreover, SM-rich (1%) diet significantly increases plasma SM levels, LDL aggregation and atherosclerotic lesions in LDL receptor KO mice.[86] All these data suggest that plasma SM plays a critical role in the development of atherosclerosis. SM on LDL retained in atherosclerotic lesions is hydrolyzed by an arterial wall sphingomyelinase, which promotes aggregation by converting SM to ceramide[51,76] (Fig. 3).

There are two ways of preventing this atherogenic event, the first being to reduce sphingomyelinase levels. Indeed, it has recently been reported that apoE KO mice lacking sphingomyelinase have decreased development of early atherosclerotic lesions and, more important, decreased retention of atherogenic lipoproteins compared with apoE KO matched for similar lipoprotein levels.[87] The second way of preventing atherogenicity is by reducing SM levels in the atherogenic lipoproteins through inhibition of the SM biosynthesis pathway in the lipoprotein-producing tissues such as the liver and small intestine.

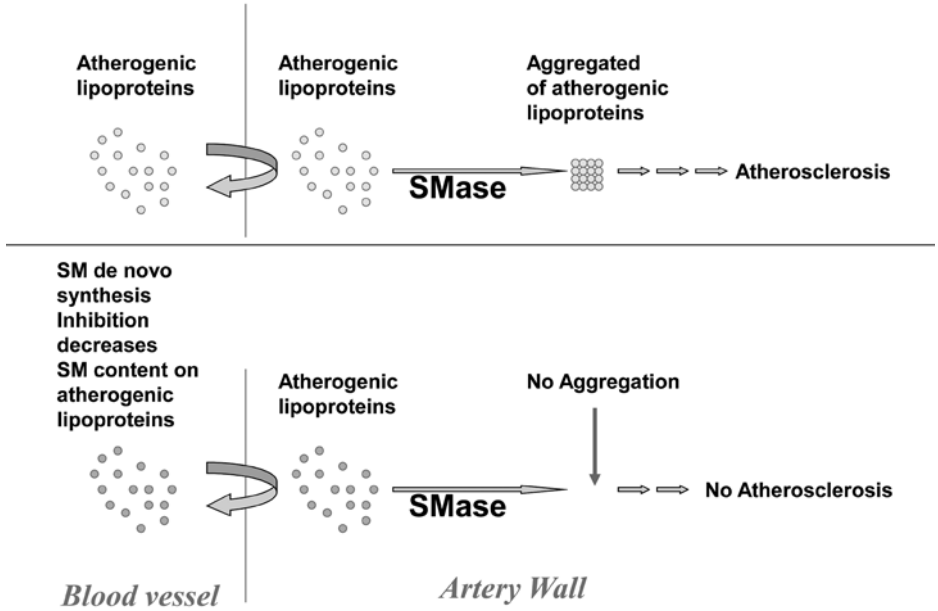

Figure 3. Role of sphingomyelin in aggregation of atherogenic lipoprotein. Generation of ceramide by sphingomyelinase aggregates intimal atherogenic lipoproteins and leads to early atherogenesis. Inhibition of SM de novo synthesis decreases sphingomyelinase substrate SM, thus decreasing aggregation of intimal lipoproteins.

SM and Cholesterol Metabolism

Given that all pathways of cholesterol efflux require cholesterol transport to the plasma membranes, the identification of molecules mediating or regulating this transport process is an important goal. Leventhal et al,[88] reported that lysosomal sphingomyelinase is involved in cholesterol transport from lysosomes to the plasma membranes. Sphingomyelinase hydrolyzes SM in late endosomes and lysosomes.[89] Because SM avidly binds cholesterol,[90,91] SM hydrolysis by sphingomyelinase enables cholesterol transport through preventing cholesterol sequestration by SM. Interestingly, humans with sphingomyelinase deficiency (Types A and B Niemann-Pick disease) have low plasma HDL cholesterol levels,[92] which could result from defective cholesterol efflux.[93]

In macrophages, ABCA1 exports cholesterol and phospholipid to lipid-free apolipoproteins, while ABCG1 and SR-BI export cholesterol to phospholipid-containing acceptors.[94] ABCA1-dependent cholesterol export involves an initial interaction of apoA-I with lipid raft membrane domains.[95,96] ABCA1 expression causes a change in overall lipid packing of the plasma membrane, probably through its ATPase-related functions. Such reorganization by ABCA1 effectively expands the nonraft membrane fractions and consequently preconditions cells for cholesterol efflux.[97] ABCG1 exports cholesterol to

HDL and other phospholipid-containing acceptors. These include particles generated during lipidation of apoA-I by ABCA1, suggesting that the two transporters cooperate in cholesterol export.[98] ABCG1 is mainly found intracellularly in the basal state, with little cell surface presentation. But on stimulation, for example by liver X receptor (LXR) agonist treatment, ABCG1 redistributes to the plasma membranes and increases cholesterol mass efflux to HDL.[99] SR-BI facilitates cholesterol efflux from macrophages.[100] Inactivation of macrophage SR-BI promotes atherosclerotic lesion development in apoE KO mice.[101] In the liver, ABCA1 makes a major contribution to the HDL in the circulation. Liver-specific ABCA1 KO mice decrease plasma HDL by about 80% compared with controls.[102,103] SR-BI is a multifunctional receptor capable of binding a wide array of native and modified lipoproteins. SRBI is abundantly expressed in liver and steroidogenic tissues, where it mediates the selective uptake of cholesteryl esters from HDL.[104,105] A definitive role for SR-BI in HDL metabolism and reverse cholesterol transport in vivo has been demonstrated using different transgenic and knockout mouse models. ABCG1 is also expressed in the liver,[106] but its function in the liver is still not well characterized.

The apical membranes of intestinal cells are enriched in SM and cholesterol,[107] indicative of the presence of lipid rafts.[108,109] Lipid rafts on the enterocyte apical membrane play an important role in cholesterol absorption and trafficking.[110,111]

ABCA1, ABCG1, and SR-BI are located in the plasma membrane rafts (SR-BI),[112,113] or associated with membrane lipid rafts (ABCA1 and ABCG1).[94-97,114] It is therefore conceivable that fundamental changes in SM levels of the plasma membranes influence the functions of these proteins and alter cholesterol homeostasis. In peripheral tissues, SM regulation of reverse cholesterol transport proteins could affect atherosclerosis. Enhanced apoAI-dependent cholesterol efflux by ABCA1 from SM-deficient Chinese hamster ovary (CHO) cells has been reported.[115]

Serine Palmitoyltransferase (SPT) and Atherosclerosis

Plasma SM and other sphingolipid intermediates play important roles in the development of coronary artery disease. It is conceivable that regulation of SM biosynthesis can alter SM levels in the plasma and on the membrane, thus influencing the process of atherosclerosis. How is SM synthesis regulated in vivo and which steps are critical?

SPT Inhibitors

Myriocin, sphingofungins and lipoxamycin are potent and highly selective naturally occurring inhibitors of SPT, inhibiting fungal and mammalian SPT in cell-free preparations with IC_{50} values in the nanomolar range[116-118] (Fig. 4). Structurally they resemble the transient intermediate postulated to form in the condensation of L-serine and palmitoyl CoA. Consistent with this, the inhibitory activity of sphingofungin B is highly dependent on its stereochemistry.[119] Myriocin-linked resins bind the Sptlc1/Sptlc2 complex tightly.[120] All the inhibitors significantly inhibit SM accumulation in both cultured cells and in vivo.[116-118]

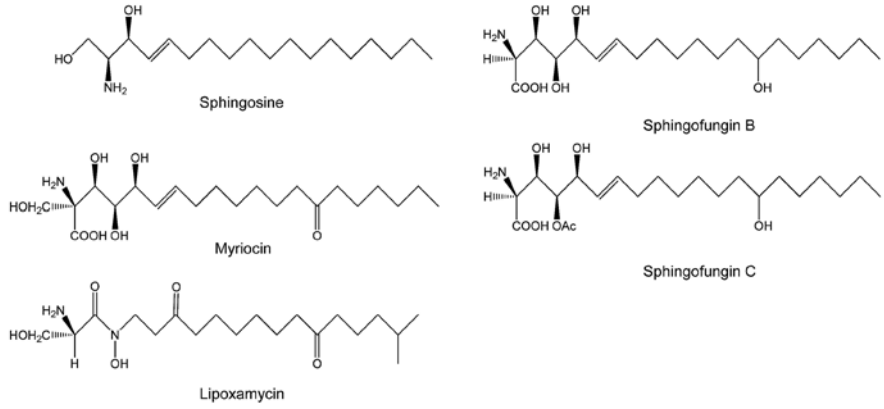

Figure 4. Structures of sphingosine and inhibitors of serine palmitoyltransferase.

The Effect of SPT Inhibition in Mouse Atherosclerosis

Park et al[7] and Hojjatti et al[8] have reported that myriocin treatment (oral administration and intraperitoneal injection, respectively) decreases plasma SM levels and atherosclerosis in apoE KO mice. Myriocin treatment also can induce lower lipid levels in apoE KO mice.[121] However, both administration methods led to reduced atherosclerosis, whereas only oral administration of myriocin lowered plasma cholesterol levels.[7] Oral administration may reduce cholesterol absorption in small intestine. When wild type and apoE KO animals were treated with myriocin, the mice absorbed significantly less cholesterol than controls with no observable pathological changes in the small intestine. Myriocin treatment also increased insulin sensitivity.[11] Thus, myriocin has direct anti-atherosclerosis vascular effects and also has the potential to function as a plasma lipid-lowering agent.

Homozygous Sptlc1 and Sptlc2 KO mice are embryonic lethal, whereas heterozygous versions of both animals, Sptlc1$^{+/-}$ and Sptlc2$^{+/-}$, are healthy. Compared with wild type mice, Sptlc1$^{+/-}$ and Sptlc2$^{+/-}$ mice had: (1) decreased liver Sptlc1 and Sptlc2 mRNA by 44% and 57%, respectively and (2) decreased liver SPT activity by 45% and 60%, respectively. However, these animals had no change in plasma SM, triglyceride, total cholesterol, phospholipids and liver SM levels.[123]

Heterozygous Sptlc1 (a subunit of SPT) knockout mice also absorbed significantly less cholesterol than controls. To understand the mechanism, the protein levels of Niemann-Pick C1-like 1 (NPC1L1), ABCG5 and ABCA1, three key factors involved in intestinal cholesterol absorption were measured. NPC1L1 and ABCA1 were decreased, whereas ABCG5 was increased in the SPT deficient small intestine. SM levels on the apical membrane were also measured and they were significantly decreased in SPT deficient mice, compared with controls.[122] These results suggested that SPT deficiency might reduce intestinal cholesterol absorption by altering NPC1L1 and ABCG5 protein levels in the apical membranes of enterocytes through lowering apical membrane SM levels. Thus, manipulation of SPT activity could provide a novel alternative treatment for dyslipidemia.

Regulation of SPT

A. Transcriptional and posttranscriptional regulation: Although the mechanism underlying the regulation of SPT is largely unknown, studies described below have begun to provide insight into transcriptional and posttranscriptional regulation. Sptlc1 and Sptlc2 mRNA and SPT enzyme activity levels increase in response to several types of inflammatory and stress stimuli. Intraperitoneal administration of endotoxin to Syrian hamsters stimulates SPT activity 2 to 3-fold in the liver, spleen and kidney, with a concomitant increase in the levels of Sptlc2 mRNA and SM.[124,125] Similar changes are observed upon administration of interleukin 1β (IL-1), an inflammatory cytokine.[124] Irradiation of epidermal cells with ultraviolet light also affects Sptlc1 and Sptlc2 mRNA levels.[126] The expression of Sptlc1 mRNA is also up-regulated in the pancreatic islets of leptin receptor-deficient obese *fa*/*fa* rats. This is suggested to be a response to an increase in intracellular fatty acid.[127]
B. Activity regulation: Consistent with the ubiquitous presence of SM in mammalian cells, SPT enzyme activity has been detected in many tissues and cell preparations.[128] The levels of SPT activity varies with development. For example, in rat lung, SPT activity increases progressively from the fetal to neonatal period and reaches a plateau at the adult stage.[129] The levels of SPT activity in several animal tissues are affected by diet.[130,131] A major mode of regulation of SPT activity occurs through substrate supply. It has been reported that the greatest activity was obtained with palmitoyl-CoA supply.[132] Pentadecanoyl-and heptadecanoyl-CoAs are also effective. In mammalian cells, palmitoyl CoA is one of the most abundant acyl-CoA types. Therefore, palmitoyl CoA is the predominant acyl-CoA substrate of SPT in vivo.[133]

Sphingomyelin Synthase (SMS) and Atherosclerosis

SMS is the last enzyme for SM biosynthesis. Its activity directly influences SM, PC and ceramide, as well as diacylglycerol (DAG) levels.[12] Manipulating SMS activity also can regulate plasma and membrane SM levels.

The Effect of SMS2 Overexpression and Deficiency on Mouse Lipoprotein Metabalism

To evaluate the in vivo role of SMS2 in SM metabolism, SMS2 KO and SMS2 liver-specific transgenic (LTg) mice were created and their plasma SM and lipoprotein metabolism were characterized.[134] On a chow diet, SMS2 KO mice had reduced plasma SM levels, but no significant changes in total cholesterol, total phospholipids, or triglyceride, compared with wild type littermates. On a high-fat diet, SMS2 KO mice showed a significant decrease in plasma SM levels, whereas SMS2LTg mice showed a significant increase in those levels, but no significant changes in other lipids. Atherogenic lipoproteins from SMS2LTg mice displayed a significantly stronger tendency toward aggregation after mammalian sphingomyelinase treatment, compared with controls. Moreover, SMS2 deficiency significantly increased plasma apoE levels

(2.0-fold), whereas liver-specific SMS2 overexpression significantly decreased those levels (1.8-fold). Finally, SMS2 KO mouse plasma promoted cholesterol efflux from macrophages, whereas SMS2LTg mouse plasma prevented it.[134] These results indicated that SMS2 is one of the determinants for plasma and liver SM levels in mice.

SMS and Apoptosis

It is believed that disordered apoptosis may occur in atherogenesis, leading to death of lipid-rich foam cells, promoting lipid core formation.[135] In two previous papers, investigators reported controversial results regarding the relationship between SMS activity and cell apoptosis. Van der Luit et al[33] reported that SMS1 gene knockdown alters the plasma membrane raft structure and reduces the internalization of alkyl-lysophospholipid, thereby reducing cell apoptosis rates. Separovic et al[136] reported that SMS1 overexpression suppresses cell apoptosis mediated by photo damage; however, they did not show the effect of SMS1 overexpression on membrane lipid rafts. Based on previous results,[137] it is believed that SMS1 and SMS2 overexpression increases SM levels in the lipid rafts on plasma membranes and promotes a more external appearance for TNFα receptor 1 (a well-known receptor in lipid rafts)[138] following TNFα stimulation, thereby promoting CHO cell apoptosis. SMS1 and SMS2 knockdown by siRNA reduces SM levels in lipid rafts on the plasma membrane and reduces the number of TLR4 (which are also well known for their presence in lipid rafts)[139] that are presented on the plasma membrane after LPS stimulation, thereby reducing macrophage apoptosis. Indeed, after LPS stimulation, SMS1/SMS2 knockdown macrophages contained significantly less TLR4 on the cell surface than control macrophages.[137]

Manipulation of SMS activity alters cellular DAG levels and, thus, may also contribute to apoptosis. Cerbon et al demonstrated that pharmacological inhibition of SMS reduces cellular DAG levels and PKC activity.[140] SMS1 or SMS2 overexpression significantly increases DAG levels in CHO cells, while SMS1 or SMS2 gene knockdown significantly reduces DAG levels in THP-1-derived macrophages.[137] DAG can regulate both conventional and novel PKCs,[141] a family of serine/threonine kinases that regulate a diverse set of cellular processes, including proapoptotic and pro-survival processes. PKCδ is generally considered a growth inhibitory or proapoptotic PKC,[142,143] while PKCε is considered a pro-survival factor.[142,144] It is possible that in both CHO cells and THP-1–derived macrophages, regulation of SMS1 or SMS2 activity by either the overexpression of their genes or gene knockdown could modulate DAG-mediated PKC activity, thereby influencing cell apoptosis.[137]

Manipulation of SMS activity also alters cellular ceramide levels and this may also contribute to apoptosis. Overexpression of both SMS1 and SMS2 is accompanied by increased levels of ceramide, as well as SM.[137] Separovic et al reported the same phenomenon when they overexpressed SMS1 in Jurkat cells.[136] This may be due the complexity of this enzyme which can catalyze bidirectional reaction.[28] The ceramide:SM ratio increases in cells that overexpress SMS compared with controls.[137] This may represent another mechanism for the increased apoptotic potential of these cells, given that ceramide is a bioactive lipid that is well known for promoting cell apoptosis.[145,146] However, ceramide levels did not change in THPderived macrophages after they were exposed to SMS siRNA.[137] This indicates that the ceramide level might not be important in SMS-knockdown–induced macrophage apoptosis.

SMS and NFκB and MAP Kinase Activation

The accumulation of macrophage-derived foam cells in the vessel wall is always accompanied by the production of a wide range of chemokines, cytokines and growth factors.[147] These factors regulate the turnover and differentiation of immigrating and resident cells, eventually influencing plaque development. One of the key regulators of inflammation is NFκB,[148] which has long been regarded as an atherogenic factor, mainly because of its regulation of many of the inflammatory genes linked to atherosclerosis.[149,150]

Depletion of cholesterol from rafts causes a redistribution of TNFα receptor 1 to nonraft plasma membrane, preventing NFκB activation[151] or ligand-induced RhoA activation[152] and such treatment also inhibits inflammatory signals mediated by TLRs.[139] Studies also suggest that NFκB activation is triggered by SM-derived ceramide.[153] On the contrary, it has been also shown that ceramide is not necessary or even inhibits NFκB activation.[154] Luberto et al[155] found that D609, a nonspecific SMS inhibitor, blocks TNFα-and phorbol ester-mediated NFκB activation that was concomitant with decreased levels of SM and DAG. This did not affect the generation of ceramide, suggesting SM and DAG derived from SM synthesis are involved in NFκB activation. Hailemariam et al found that NFκB activation and its target gene expression are attenuated in macrophages from SMS2 KO mice in response to LPS stimulation and in SMS2 siRNA-treated HEK 293 cells after TNFα simulation.[156] In line with attenuated NFκB activation, it was found that SMS2 deficiency substantially diminished the abundance of toll like receptor 4 (TLR4)-MD2 complex levels on the surface of macrophages after LPS stimulation and SMS2 siRNA treatment reduced TNFα-stimulated lipid raft recruitment of TNFα receptor-1 in HEK293 cells.[156] Thus, SMS2 is a modulator of NFκB activation and could play an important role in NFκB-mediated atherogenic process (Fig. 5).

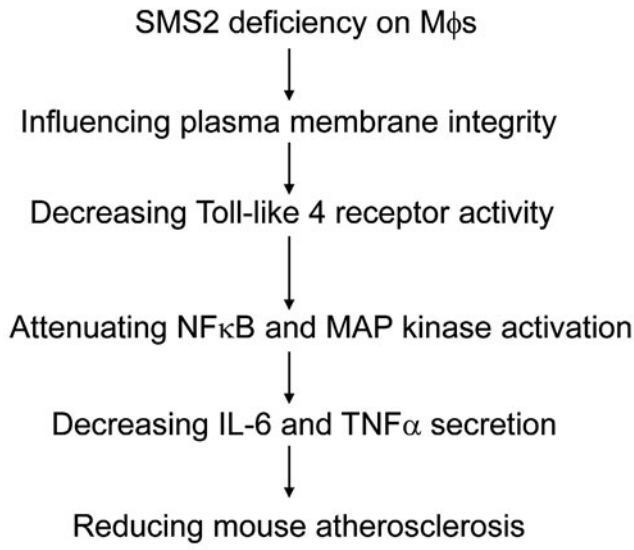

Figure 5. Mechanism of reduced atherosclerosis by deficiency of sphingomyelin synthase 2.

SMS2 deficiency may also influence signal transduction pathways other than NFκB activation. The activation of MAP kinases was attenuated in SMS2 KO macrophages.[156]

Macrophage SMS2 Deficiency and Atherosclerosis

In order to evaluate the relationship between macrophage SMS2 deficiency and atherosclerosis, we transplanted SMS2 KO mouse bone marrow into LDL receptor KO (*Ldlr*$^{-/-}$) mice (SMS2$^{-/-}$ macrophages *Ldlr*$^{-/-}$), creating a mouse model of SMS2 deficiency in the macrophages. After 3 months on a Western diet, SMS2 deficiency decreased atherosclerotic lesions in the aortic arch, root (57%, $P < 0.001$) and the entire aorta (42%, $P < 0.01$), compared with wild type macrophages transplanted into *Ldlr*$^{-/-}$ mice. Analysis of plaque morphology revealed that SMS2 macrophage deficiency produced less necrotic core area (71%, $P < 0.001$) and more collagen content (35%, $P < 0.05$) in atherosclerotic lesions and less free cholesterol and cholesteryl ester levels in the brachiocephalic artery. Therefore, SMS2 deficiency in the macrophages reduces atherosclerosis in mice. Macrophage SMS2 is thus a potential therapeutic target for treatment of this disease[9] (Fig. 5).

CERAMIDE IN LIPOTOXIC CARDIOMYOPATHY

Cardiomyopathy, an outcome of many chronic cardiovascular diseases that is often found in patients with diabetes, is sometimes associated with increased heart content of lipids.

Diabetic cardiomyopathy accounts for increased morbidity and mortality after myocardial infarction in diabetic compared with nondiabetic patients.[157]

The heart utilizes FFA as an important major fuel source. Over 70% of the energy needs for cardiac function comes from oxidation of FA, with the balance provided by carbohydrates and lactate.[158,159] FFAs derived from the hydrolysis of TG stored in adipose tissue bind to circulating albumin and are delivered to heart. Alternatively, TG-rich lipoproteins are internalized by receptor-mediated processes and are hydrolyzed to liberate FFA inside the cells.[160] When cardiac FA uptake is elevated due to fasting, diabetes or obesity,[161,162] excess fatty acyl CoAs and unesterified FA not used for oxidation can be stored in TG. In animal models of obesity and diabetes, TG accumulation in heart is associated with impaired contractile function; this suggests that excess TG or its metabolites are toxic.[3,163] These potentially toxic metabolites include ceramide and DAG. Ceramide is also increased in hearts of obese Zucker with impaired myocardial contractility.[3] Cardiac overexpression of PPARα and long chain acyl-CoA synthetase1 traps greater levels of FFAs in the hearts and the synthesis of ceramide are activated due to increased substrate availability for ceramide synthesis.[164]

Mice with cardiac overexpression of glycosylphosphatidylinositol membrane-anchored LpL mice (LpL_{GPI}) also have increased increased cardiac ceramide and apoptosis markers including cytosolic cytochrome c, caspase 3 expression and activity.[165] Park et al demonstrated that inhibition of ceramide biosynthesis by myriocin or heterozygous deletion of Sptlc1 leads to decreased expression of some apoptotic genes and improved cardiac contraction in LpL_{GPI}.[10] In this study, blockage of ceramide biosynthesis appears to modulate mitochondrial substrate oxidation (Fig. 6). LpL_{GPI} hearts have increased uptake of FFA and rely on FA oxidation for cardiac energy production. A potential

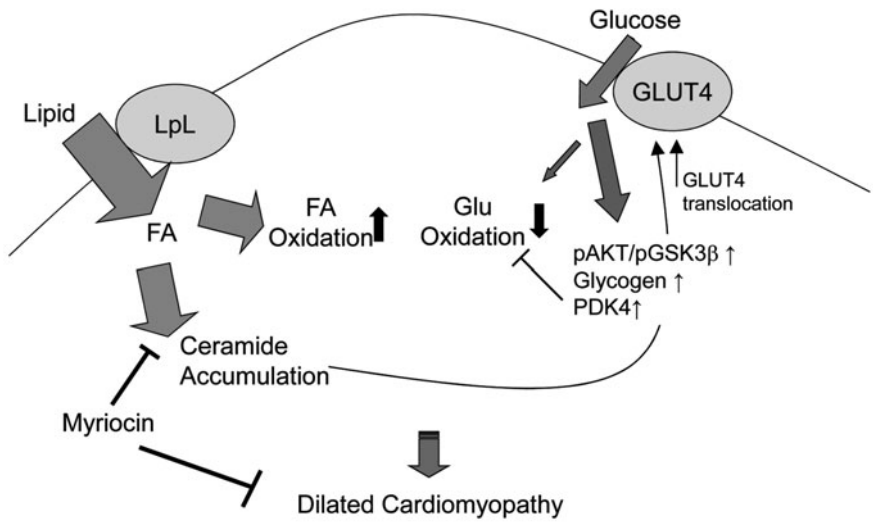

Figure 6. Role of ceramide in modulation of cardiac energetics. Increased uptake of lipids into the hearts alters the balance of glucose and fatty acid metabolism and causes dilated cardiomyopathy. Inhibition of SPT ameliorates cardiac failure by restoring cardiac function by balancing the substrate oxidation of glucose and fatty acids.

mechanism is that pharmacological and genetic inhibition of SPT upregulated pyruvate dehydrogenase kinase-4 and decreased the rate of glucose oxidation.

However, glucose uptake is increased in LpL_{GPI} hearts. This paradoxical fate of glucose is explained by accumulation of glucose in a form of glycogen with increased phosphorylated glycogen synthase 3β.[10] In isolated perfused LpL_{GPI} hearts, myriocin restored cardiac efficiency, enhancing myocardial energetics by maintaining cardiac performance at a lower oxygen cost. Even with improved cardiac function and balanced substrate utilization by myriocin treatment, a longterm treatment of LpL_{GPI} mice with myriocin only partially rescued the survival rate. A potential reason is due to involvement of other lipid metabolites in cardiac dysfunction. Altered PKC signalling pathway by DAG and toxicity of FFA are the probable candidates for cardiac failure. More studies should be followed to distinguish the role of ceramide from other lipid metabolites.

CONCLUSION

Coronary heart disease and heart failure are major causes of mortality in developed countries.

Although presently known risk factors have some predictive value for the disease, a major part of the variability in this process remains unexplained. In addition, therapy aimed at lowering LDL cholesterol reduces only a small fraction (roughly 30%) of the burden of atherosclerotic disease. It is extremely important to find new approaches for better understanding of the disease and for treating it. Exploration of the SM biosynthesis pathway is one such approach. SM is implicated as a biochemical modulator of lipoprotein metabolism and atherosclerosis and ceramide is suggested as a metabolic switch

determining substrate preference for cardiac energetics. One highly important aspect of the reported work is the discovery that inhibiting sphingolipid biosynthesis may have great therapeutic value for the treatment of atherosclerosis and cardiac failure. To confirm this, a prospective clinical study in human populations should be followed to evaluate whether plasma SM is a real risk factor for atherosclerosis. In addition, sphingolipid biosynthesis is tightly linked to development of insulin resistance, obesity and atherosclerosis. The mechanisms of sphingolipid biosynthesis involved in these chronic diseases need to be further elucidated in detail. Thus, sphingolipid modulation in atherosclerosis and ardiomyopathy will provide a therapeutic rationale to cure the patients inflicting with these chronic diseases.

REFERENCES

1. Shimabukuro M, Zhou YT, Levi M et al. Fatty acidinduced beta cell apoptosis: a link between obesity and diabetes. Proc Natl Acad Sci USA 1998; 95:2498-2502.
2. Koyama K, Chen G, Lee Y et al. Tissue triglycerides, insulin resistance and insulin production: implications for hyperinsulinemia of obesity. Am J Physiol 1997; 273:E708-713.
3. Zhou YT, Grayburn P, Karim A et al. Lipotoxic heart disease in obese rats: implications for human obesity. Proc Natl Acad Sci USA 2000; 97:1784-1789.
4. Dyntar D, Eppenberger-Eberhardt M, Maedler K et al. Glucose and palmitic acid induce degeneration of myofibrils and modulate apoptosis in rat adult cardiomyocytes. Diabetes 2001; 50:2105-2113.
5. Petersen KF, Shulman GI. Etiology of insulin resistance. Am J Med 2006; 119:S10-16.
6. Jiang XC, Paultre F, Pearson TA et al. Plasma sphingomyelin level as a risk factor for coronary artery disease. Arterioscler Thromb Vasc Biol 2000; 20:2614-2618.
7. Park TS, Panek RL, Mueller SB et al. Inhibition of sphingomyelin synthesis reduces atherogenesis in apolipoprotein E-knockout mice. Circulation 2004; 110:3465-3471.
8. Hojjati MR, Li Z, Zhou H et al. Effect of myriocin on plasma sphingolipid metabolism and atherosclerosis in apoE-deficient mice. J Biol Chem 2005; 280:10284-10289.
9. Liu J, Huan C, Chakraborty M et al. Macrophage sphingomyelin synthase 2 (SMS2) deficiency decreases atherosclerosis in mice. Circulation Research. 2009.
10. Park TS, Hu Y, Noh HL et al. Ceramide is a cardiotoxin in lipotoxic cardiomyopathy. J Lipid Res 2008; 49:2101-2112.
11. Holland WL, Brozinick JT, Wang LP et al. Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat- and obesityinduced insulin resistance. Cell Metab 2007; 5:167-179.
12. Merrill AH Jr, Jones DD. An update of the enzymology and regulation of sphingomyelin metabolism. Biochim Biophys Acta 1990; 1044:1-12.
13. Weiss B, Stoffel W. Human and murine serine-palmitoyl-CoA transferase—cloning, expression and characterization of the key enzyme in sphingolipid synthesis. Eur J Biochem 1997; 249:239-247.
14. Hanada K, Hara T, Nishijima M et al. A mammalian homolog of the yeast LCB1 encodes a component of serine palmitoyltransferase, the enzyme catalyzing the first step in sphingolipid synthesis. J Biol Chem 1997; 272:32108-32114.
15. Yasuda S, Nishijima M, Hanada K. Localization, topology and function of the LCB1 subunit of serine palmitoyltransferase in mammalian cells. J Biol Chem 2003; 278:4176-4183.
16. Hornemann T, Richard S, Rutti MF et al. Cloning and initial characterization of a new subunit for mammalian serine-palmitoyltransferase. J Biol Chem 2006; 281:37275-37281.
17. Gable K, Slife H, Bacikova D et al. Tsc3p is an 80-amino acid protein associated with serine palmitoyltransferase and required for optimal enzyme activity. J Biol Chem 2000; 275:7597-7603.
18. Han G, Gupta SD, Gable K et al. Identification of small subunits of mammalian serine palmitoyltransferase that confer distinct acyl-CoA substrate specificities. Proc Natl Acad Sci USA 2009; 106:8186-8191.
19. Lipsky NG, Pagano RE. Intracellular translocation of fluorescent sphingolipids in cultured fibroblasts: endogenously synthesized sphingomyelin and glucocerebroside analogues pass through the Golgi apparatus en route to the plasma membrane. J Cell Biol 1985; 100:27-34.
20. Kobayashi T, Pagano RE. Lipid transport during mitosis. Alternative pathways for delivery of newly synthesized lipids to the cell surface. J Biol Chem 1989; 264:5966-5973.
21. Futerman AH, Stieger B, Hubbard AL et al. Sphingomyelin synthesis in rat liver occurs predominantly at the cis and medial cisternae of the Golgi apparatus. J Biol Chem 1990; 265:8650-8657.

22. Marggraf WD, Zertani R, Anderer FA et al. The role of endogenous phosphatidylcholine and ceramide in the biosynthesis of sphingomyelin in mouse fibroblasts. Biochim Biophys Acta 1982; 710:314-323.
23. Malgat M, Maurice A, Baraud J. Sphingomyelin and ceramidephosphoethanolamine synthesis by microsomes and plasma membranes from rat liver and brain. J Lipid Res 1986; 27:251-260.
24. van Helvoort A, van't Hof W, Ritsema T et al. Conversion of diacylglycerol to phosphatidylcholine on the basolateral surface of epithelial (Madin-Darby canine kidney) cells. Evidence for the reverse action of a sphingomyelin synthase. J Biol Chem 1994; 269:1763-1769.
25. Allan D, Obradors MJ. Enzyme distributions in subcellular fractions of BHK cells infected with Semliki forest virus: evidence for a major fraction of sphingomyelin synthase in the trans-golgi network. Biochim Biophys Acta 1999; 1450:277-287.
26. Albi E, Magni MV. Sphingomyelin synthase in rat liver nuclear membrane and chromatin. FEBS Lett 1999; 460:369-372.
27. Albi E, MM. Chromatin-associated sphingomyelin: metabolism in relation to cell function. Cell Biochem Funct 2003; 21:211-215.
28. Huitema K, van den Dikkenberg J, Brouwers JF et al. Identification of a family of animal sphingomyelin synthases. Embo J 2004; 23:33-44.
29. Yamaoka S, Miyaji M, Kitano T et al. Expression cloning of a human cDNA restoring sphingomyelin synthesis and cell growth in sphingomyelin synthase-defective lymphoid cells. J Biol Chem 2004; 279:18688-18693.
30. Yeang C, Varshney S, Wang R et al. The domain responsible for sphingomyelin synthase (SMS) activity. Biochim Biophys Acta 2008; 1781:610-617.
31. Li Z, Hailemariam TK, Zhou H et al. Inhibition of sphingomyelin synthase (SMS) affects intracellular sphingomyelin accumulation and plasma membrane lipid organization. Biochim Biophys Acta 2007; 1771:1186-1194.
32. Miyaji M, Jin ZX, Yamaoka S et al. Role of membrane sphingomyelin and ceramide in platform formation for Fasmediated apoptosis. J Exp Med 2005; 202:249-259.
33. Van der Luit AH, Budde M, Zerp S et al. Resistance to alkyl-lysophospholipid induced apoptosis due to downregulated sphingomyelin synthase 1 expression with consequent sphingomyelin- and cholesterol-deficiency in lipid rafts. Biochem J 2007; 401:541-549.
34. Merkel M, Eckel RH Goldberg IJ. Lipoprotein lipase: genetics, lipid uptake and regulation. J Lipid Res 2002; 43:1997-2006.
35. Otarod JK, Goldberg IJ. Lipoprotein lipase and its role in regulation of plasma lipoproteins and cardiac risk. Curr Atheroscler Rep 2004; 6:335-342.
36. Kuksis A, Breckenridge WC, Myher JJ et al. Replacement of endogenous phospholipids in rat plasma lipoproteins during intravenous infusion of an artificial lipid emulsion. Can J Biochem 1978; 56:630-639.
37. Saito H, Arimoto I, Tanaka M et al. Inhibition of lipoprotein lipase activity by sphingomyelin: role of membrane surface structure. Biochim Biophys Acta 2000; 1486:312-320.
38. Arimoto I, Saito H, Kawashima Y et al. Effects of sphingomyelin and cholesterol on lipoprotein lipase-mediated lipolysis in lipid emulsions. J Lipid Res 1998; 39:143-151.
39. Lobo LI, Wilton DC. Combined effects of sphingomyelin and cholesterol on the hydrolysis of emulsion particle triolein by lipoprotein lipase. Biochim Biophys Acta 1997; 1349:122-130.
40. Cantin B, Brun LD, Gagne C et al. Alterations in erythrocyte membrane lipid composition and fluidity in primary lipoprotein lipase deficiency. Biochim Biophys Acta 1992; 1139:25-31.
41. Mahley RW, Ji ZS. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. J Lipid Res 1999; 40:1-16.
42. Kothapalli D, Fuki I, Ali K et al. Antimitogenic effects of HDL and APOE mediated by Cox-2-dependent IP activation. J Clin Invest 2004; 113:609-618.
43. Huang Y, Ji ZS, Brecht WJ et al. Overexpression of apolipoprotein E3 in transgenic rabbits causes combined hyperlipidemia by stimulating hepatic VLDL production and impairing VLDL lipolysis. Arterioscler Thromb Vasc Biol 1999; 19:2952-2959.
44. Mensenkamp AR, Jong MC, van Goor H et al. Apolipoprotein E participates in the regulation of very low density lipoprotein-triglyceride secretion by the liver. J Biol Chem 1999; 274:35711-35718.
45. Mahley RW, Huang Y, Weisgraber KH. Putting cholesterol in its place: apoE and reverse cholesterol transport. J Clin Invest 2006; 116:1226-1229.
46. Arimoto I, Matsumoto C, Tanaka M et al. Surface composition regulates clearance from plasma and triolein lipolysis of lipid emulsions. Lipids 1998; 33:773-779.
47. Morita SY, Nakano M, Sakurai A et al. Formation of ceramide-enriched domains in lipid particles enhances the binding of apolipoprotein E. FEBS Lett 2005; 579:1759-1764.
48. Morita SY, Okuhira K, Tsuchimoto N et al. Effects of sphingomyelin on apolipoprotein E- and lipoprotein lipase-mediated cell uptake of lipid particles. Biochim Biophys Acta 2003; 1631:169-176.

49. Lucic D, Huang ZH, Gu de S et al. Regulation of macrophage apoE secretion and sterol efflux by the LDL receptor. J Lipid Res 2007; 48:366-372.
50. Schlitt A, Hojjati MR, von Gizycki H et al. Serum sphingomyelin levels are related to the clearance of postprandial remnant-like particles. J Lipid Res 2005; 46:196-200.
51. Jeong T, Schissel SL, Tabas I et al. 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 1998; 101:905-912.
52. Krieger M. Charting the fate of the "good cholesterol": identification and characterization of the high-density lipoprotein receptor SR-BI. Annu Rev Biochem 1999; 68:523-558.
53. Van Eck M, Pennings M, Hoekstra M et al. Scavenger receptor BI and ATP-binding cassette transporter A1 in reverse cholesterol transport and atherosclerosis. Curr Opin Lipidol 2005; 16:307-315.
54. Barter PJ, Brewer HB Jr, Chapman MJ et al. Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis. Arterioscler Thromb Vasc Biol 2003; 23:160-167.
55. Subbaiah PV, Liu M. Role of sphingomyelin in the regulation of cholesterol esterification in the plasma lipoproteins. Inhibition of lecithincholesterol acyltransferase reaction. J Biol Chem 1993; 268:20156-20163.
56. Bolin DJ, Jonas A. Sphingomyelin inhibits the lecithin-cholesterol acyltransferase reaction with reconstituted high density lipoproteins by decreasing enzyme binding. J Biol Chem 1996; 271:19152-19158.
57. Rye KA, Hime NJ, Barter PJ. The influence of sphingomyelin on the structure and function of reconstituted high density lipoproteins. J Biol Chem 1996; 271:4243-4250.
58. Tall AR. Cholesterol efflux pathways and other potential mechanisms involved in the athero-protective effect of high density lipoproteins. J Intern Med 2008; 263:256-273.
59. Subbaiah PV, Gesquiere LR, Wang K. Regulation of the selective uptake of cholesteryl esters from high density lipoproteins by sphingomyelin. J Lipid Res 2005; 46:2699-2705.
60. Marques-Vidal P, Jauhiainen M, Metso J et al. Transformation of high density lipoprotein 2 particles by hepatic lipase and phospholipid transfer protein. Atherosclerosis 1997; 133:87-95.
61. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993; 362:801-809.
62. Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 1991; 88:1785-1792.
63. Yla-Herttuala S, Palinski W, Rosenfeld ME et al. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. J Clin Invest 1989; 84:1086-1095.
64. Williams KJ, Tabas I. The response-to-retention hypothesis of early atherogenesis. Arterioscler Thromb Vasc Biol 1995; 15:551-561.
65. Williams KJ, Tabas I. The response-to-retention hypothesis of atherogenesis reinforced. Curr Opin Lipidol 1998; 9:471-474.
66. Tabas I, Williams KJ, Boren J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. Circulation 2007; 116:1832-1844.
67. Nievelstein PF, Fogelman AM, Mottino G et al. Lipid accumulation in rabbit aortic intima 2 hours after bolus infusion of low density lipoprotein. A deep-etch and immunolocalization study of ultrarapidly frozen tissue. Arterioscler Thromb 1991; 11:1795-1805.
68. Smith EB. Intimal and medial lipids in human aortas. Lancet 1960; 1:799-803.
69. Newman HA, Mc CE, Zilversmit DB. The synthesis of C14-lipids in rabbit atheromatous lesions. J Biol Chem 1961; 236:1264-1268.
70. Phillips GB, Dodge JT. Composition of phospholipids and of phospholipid fatty acids of human plasma. J Lipid Res 1967; 8:676-681.
71. Portman OW, Illingworth DR. Arterial metabolism in primates. Primates Med 1976; 9:145-223.
72. Hakomori S. Glycosphingolipids in cellular interaction, differentiation, and oncogenesis. Annu Rev Biochem 1981; 50:733-764.
73. Kummerow FA, Cook LS, Wasowicz E et al. Changes in the phospholipid composition of the arterial cell can result in severe atherosclerotic lesions. J Nutr Biochem 2001; 12:602-607.
74. Hoff HF, Morton RE. Lipoproteins containing apo B extracted from human aortas. Structure and function. Ann N Y Acad Sci 1985; 454:183-194.
75. Guyton JR, Klemp KF. Development of the lipid-rich core in human atherosclerosis. Arterioscler Thromb Vasc Biol 1996; 16:4-11.
76. Schissel SL, Tweedie-Hardman J, Rapp JH et al. Rabbit aorta and human atherosclerotic lesions hydrolyze the sphingomyelin of retained low-density lipoprotein. Proposed role for arterial-wall sphingomyelinase in subendothelial retention and aggregation of atherogenic lipoproteins. J Clin Invest 1996; 98:1455-1464.
77. Schissel SL, Jiang X, Tweedie-Hardman J et al. Secretory sphingomyelinase, a product of the acid sphingomyelinase gene, can hydrolyze atherogenic lipoproteins at neutral pH. Implications for atherosclerotic lesion development. J Biol Chem 1998; 273:2738-2746.
78. Zilversmit DB, Mc CE, Jordan PH et al. The synthesis of phospholipids in human atheromatous lesions. Circulation 1961; 23:370-375.

79. Eisenberg S, Stein Y, Stein O. Phospholipases in arterial tissue. IV. The role of phosphatide acyl hydrolase, lysophosphatide acyl hydrolase, and sphingomyelin choline phosphohydrolase in the regulation of phospholipid composition in the normal human aorta with age. J Clin Invest 1969; 48:2320-2329.
80. Okwu AK, Xu XX, Shiratori Y et al. Regulation of the threshold for lipoprotein-induced acyl-CoA:cholesterol O-acyltransferase stimulation in macrophages by cellular sphingomyelin content. J Lipid Res 1994; 35:644-655.
81. Noel C, Marcel YL, Davignon J. Plasma phospholipids in the different types of primary hyperlipoproteinemia. J Lab Clin Med 1972; 79:611-621.
82. Rodriguez JL, Ghiselli GC, Torreggiani D et al. Very low density lipoproteins in normal and cholesterol-fed rabbits: lipid and protein composition and metabolism. Part 1. Chemical composition of very low density lipoproteins in rabbits. Atherosclerosis 1976; 23:73-83.
83. Zhang SH, Reddick RL, Piedrahita JA et al. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. Science 1992; 258:468-471.
84. Plump AS, Smith JD, Hayek T et al. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell 1992; 71:343-353.
85. Schlitt A, Blankenberg S, Yan D et al. Further evaluation of plasma sphingomyelin levels as a risk factor for coronary artery disease. Nutr Metab (Lond) 2006; 3:5.
86. Li Z, Basterr MJ, Hailemariam TK et al. The effect of dietary sphingolipids on plasma sphingomyelin metabolism and atherosclerosis. Biochim Biophys Acta 2005; 1735:130-134.
87. Devlin CM, Leventhal AR, Kuriakose G et al. Acid sphingomyelinase promotes lipoprotein retention within early atheromata and accelerates lesion progression. Arterioscler Thromb Vasc Biol 2008; 28:1723-1730.
88. Leventhal AR, Chen W, Tall AR et al. Acid sphingomyelinase-deficient macrophages have defective cholesterol trafficking and efflux. J Biol Chem 2001; 276:44976-44983.
89. Schuchman EH, Desnick RJ. The Metabolic and Molecular Basis of Inherited Disease (Scriver CR, Beaudet AL, Sly WS and Valle D eds), McGraw-Hill, New York 1995; 2601-2624.
90. Slotte JP. Sphingomyelin-cholesterol interactions in biological and model membranes. Chem Phys Lipids 1999; 102:13-27.
91. Ridgway ND. Interactions between metabolism and intracellular distribution of cholesterol and sphingomyelin. Biochim Biophys Acta 2000; 1484:129-141.
92. Viana MB, Giugliani R, Leite VH et al. Very low levels of high density lipoprotein cholesterol in four sibs of a family with nonneuropathic Niemann-Pick disease and seablue histiocytosis. J Med Genet 1990; 27:499-504.
93. Tall AR, Wang N. Tangier disease as a test of the reverse cholesterol transport hypothesis. J Clin Invest 2000; 106:1205-1207.
94. Jessup W, Gelissen IC, Gaus K et al. Roles of ATP binding cassette transporters A1 and G1, scavenger receptor BI and membrane lipid domains in cholesterol export from macrophages. Curr Opin Lipidol 2006; 17:247-257.
95. Gaus K, Kritharides L, Schmitz G et al. Apolipoprotein A-1 interaction with plasma membrane lipid rafts controls cholesterol export from macrophages. FASEB J 2004; 18:574-576.
96. Mendez AJ, Lin G, Wade DP et al. Membrane lipid domains distinct from cholesterol/sphingomyelin-rich rafts are involved in the ABCA1-mediated lipid secretory pathway. J Biol Chem 2001; 276:3158-3166.
97. Landry YD, Denis M, Nandi S et al. ATP-binding cassette transporter A1 expression disrupts raft membrane microdomains through its ATPase-related functions. J Biol Chem 2006; 281:36091-36101.
98. Gelissen IC, Harris M, Rye KA et al. ABCA1 and ABCG1 synergize to mediate cholesterol export to apoA-I. Arterioscler Thromb Vasc Biol 2006; 26:534-540.
99. Wang N, Ranalletta M, Matsuura F et al. LXRinduced redistribution of ABCG1 to plasma membrane in macrophages enhances cholesterol mass efflux to HDL. Arterioscler Thromb Vasc Biol 2006; 26:1310-1316.
100. Huang ZH, Gu D, Lange Y et al. Expression of scavenger receptor BI facilitates sterol movement between the plasma membrane and the endoplasmic reticulum in macrophages. Biochemistry 2003; 42:3949-3955.
101. Zhang W, Yancey PG, Su YR et al. Inactivation of macrophage scavenger receptor class B type I promotes atherosclerotic lesion development in apolipoprotein Edeficient mice. Circulation 2003; 108:2258-2263.
102. Timmins JM, Lee JY, Boudyguina E et al. Targeted inactivation of hepatic Abca1 causes profound hypoalphalipoproteinemia and kidney hypercatabolism of apoA-I. J Clin Invest 2005; 115:1333-1342.
103. Lee JY, Parks JS. ATP-binding cassette transporter AI and its role in HDL formation. Curr Opin Lipidol 2005; 16:19-25.
104. Landschulz KT, Pathak RK, Rigotti A et al. Regulation of scavenger receptor, class B, type I, a high density lipoprotein receptor, in liver and steroidogenic tissues of the rat. J Clin Invest 1996; 98:984-995.
105. Stangl H, Hyatt M, Hobbs HH. Transport of lipids from high and low density lipoproteins via scavenger receptor-BI. J Biol Chem 1999; 274:32692-32698.
106. Kennedy MA, Barrera GC, Nakamura K et al. ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. Cell Metab 2005; 1:121-131.

107. Christiansen K, Carlsen J. Microvillus membrane vesicles from pig small intestine. Purity and lipid composition. Biochim Biophys Acta 1981; 647:188-195.
108. Danielsen EM, Hansen GH. Lipid raft organization and function in the small intestinal brush border. J Physiol Biochem 2008; 64:377-382.
109. Brown DA, Rose JK. Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. Cell 1992; 68:533-544.
110. Field FJ, Born E, Murthy S et al. Caveolin is present in intestinal cells: role in cholesterol trafficking? J Lipid Res 1998; 39:1938-1950.
111. Garmy N, Taieb N, Yahi N et al. Interaction of cholesterol with sphingosine: physicochemical characterization and impact on intestinal absorption. J Lipid Res 2005; 46:36-45.
112. Graf GA, Connell PM, van der Westhuyzen DR et al. The class B, type I scavenger receptor promotes the selective uptake of high density lipoprotein cholesterol ethers into caveolae. J Biol Chem 1999; 274:12043-12048.
113. Babitt J, Trigatti B, Rigotti A et al. Murine SR-BI, a high density lipoprotein receptor that mediates selective lipid uptake, is N-glycosylated and fatty acylated and colocalizes with plasma membrane caveolae. J Biol Chem 1997; 272:13242-13249.
114. Drobnik W, Borsukova H, Bottcher A et al. Apo AI/ABCA1-dependent and HDL3-mediated lipid efflux from compositionally distinct cholesterol-based microdomains. Traffic 2002; 3:268-278.
115. Nagao K, Takahashi K, Hanada K et al. Enhanced apoA-I-dependent cholesterol efflux by ABCA1 from sphingomyelin-deficient Chinese hamster ovary cells. J Biol Chem 2007; 282:14868-14874.
116. Zweerink MM, Edison AM, Wells GB et al. Characterization of a novel, potent and specific inhibitor of serine palmitoyltransferase. J Biol Chem 1992; 267:25032-25038.
117. Mandala SM, Frommer BR, Thornton RA et al. Inhibition of serine palmitoyl-transferase activity by lipoxamycin. J Antibiot (Tokyo) 1994; 47:376-379.
118. Miyake Y, Kozutsumi Y, Nakamura S et al. Serine palmitoyltransferase is the primary target of a sphingosine-like immunosuppressant, ISP-1/myriocin. Biochem Biophys Res Commun 1995; 211:396-403.
119. Kobayashi SFT, Hayashi T, Nishijima M et al. Catalytic asymmetric syntheses of antifungal sphingofungins and their biological activity as potent inhibitors of serine palmitoyltransferase (SPT). J Am Chem Soc 1998; 120:908-919.
120. Chen JK, Lane WS, Schreiber SL. The identification of myriocin-binding proteins. Chem Biol 1999; 6:221-235.
121. Park TS, Rosebury W, Kindt EK et al. Serine palmitoyltransferase inhibitor myriocin induces the regression of atherosclerotic plaques in hyperlipidemic ApoE-deficient mice. Pharmacol Res 2008; 58:45-51.
122. Li ZPT, Li Y, Pan X et al. Serine Palmitoyltransferase (SPT) Deficient Mice Absorb Less Cholesterol Biochim Biophys Acta in press. 2009.
123. Hojjati MR, Li Z, Jiang XC. Serine palmitoyl-CoA transferase (SPT) deficiency and sphingolipid levels in mice. Biochim Biophys Acta 2005; 1737:44-51.
124. Memon RA, Holleran WM, Moser AH et al. Endotoxin and cytokines increase hepatic sphingolipid biosynthesis and produce lipoproteins enriched in ceramides and sphingomyelin. Arterioscler Thromb Vasc Biol 1998; 18:1257-1265.
125. Memon RA, Holleran WM, Uchida Y et al. Regulation of sphingolipid and glycosphingolipid metabolism in extrahepatic tissues by endotoxin. J Lipid Res 2001; 42:452-459.
126. Farrell AM, Uchida Y, Nagiec MM et al. UVB irradiation up-regulates serine palmitoyltransferase in cultured human keratinocytes. J Lipid Res 1998; 39:2031-2038.
127. Shimabukuro M, Higa M, Zhou YT et al. Lipoapoptosis in beta-cells of obese prediabetic fa/fa rats. Role of serine palmitoyltransferase overexpression. J Biol Chem 1998; 273:32487-32490.
128. Dickson RC, Lester RL, Nagiec MM. Serine palmitoyltransferase. Methods Enzymol 2000; 311:3-9.
129. Longo CA, Tyler D, Mallampalli RK. Sphingomyelin metabolism is developmentally regulated in rat lung. Am J Respir Cell Mol Biol 1997; 16:605-612.
130. Geelen MJ, Beynen AC. Consumption of olive oil has opposite effects on plasma total cholesterol and sphingomyelin concentrations in rats. Br J Nutr 2000; 83:541-547
131. Rotta LN, Da Silva CG, Perry ML et al. Undernutrition decreases serine palmitoyltransferase activity in developing rat hypothalamus. Ann Nutr Metab 1999; 43:152-158.
132. Merrill AH Jr. Characterization of serine palmitoyltransferase activity in Chinese hamster overy cells. Biochim Biophys Acta 1983; 754:284-291.
133. Hanada K, Hara T, Nishijima M. Purification of the serine palmitoyltransferase complex responsible for sphingoid base synthesis by using affinity peptide chromatography techniques. J Biol Chem 2000; 275:8409-8415.
134. Liu J, Zhang H, Li Z et al. Sphingomyelin Synthase 2 Is One of the Determinants for Plasma and Liver Sphingomyelin Levels in Mice. Arterioscler Thromb Vasc Biol. 2009.

135. Geng YJ, Libby P. Progression of atheroma: a struggle between death and procreation. Arterioscler Thromb Vasc Biol 2002; 22:1370-1380.
136. Separovic D, Hanada K, Maitah MY et al. Sphingomyelin synthase 1 suppresses ceramide production and apoptosis postphotodamage. Biochem Biophys Res Commun 2007; 358:196-202.
137. Ding T, Li Z, Hailemariam T et al. SMS overexpression and knockdown: impact on cellular sphingomyelin and diacylglycerol metabolism and cell apoptosis. J Lipid Res 2008; 49:376-385.
138. Ko YG, Lee JS, Kang YS et al. TNF-alphamediated apoptosis is initiated in caveolae-like domains. J Immunol 1999; 162:7217-7223.
139. Triantafilou M, Miyake K, Golenbock DT et al. Mediators of innate immune recognition of bacteria concentrate in lipid rafts and facilitate lipopolysaccharide-induced cell activation. J Cell Sci 2002; 115:2603-2611.
140. Cerbon J, del Carmen Lopez-Sanchez R. Diacylglycerol generated during sphingomyelin synthesis is involved in protein kinase C activation and cell proliferation in Madin-Darby canine kidney cells. Biochem J 2003; 373:917-924.
141. Wakelam MJ. Diacylglycerol—when is it an intracellular messenger? Biochim Biophys Acta 1998; 1436:117-126.
142. Griner EM, Kazanietz MG. Protein kinase C and other diacylglycerol effectors in cancer. Nat Rev Cancer 2007; 7:281-294.
143. Mayor S, Maxfield FR. Insolubility and redistribution of GPIanchored proteins at the cell surface after detergent treatment. Mol Biol Cell 1995; 6:929-944.
144. Murriel CL, Mochly-Rosen D. Opposing roles of delta and epsilonPKC in cardiac ischemia and reperfusion: targeting the apoptotic machinery. Arch Biochem Biophys 2003; 420:246-254.
145. Hannun YA, Luberto C. Ceramide in the eukaryotic stress response. Trends Cell Biol 2000; 10:73-80.
146. Kolesnick R. The therapeutic potential of modulating the ceramide/sphingomyelin pathway. J Clin Invest 2002; 110:3-8.
147. Libby P. Inflammation in atherosclerosis. Nature 2002; 420:868-874.
148. Mayo MW, Baldwin AS. The transcription factor NF-kappaB: control of oncogenesis and cancer therapy resistance. Biochim Biophys Acta 2000; 1470:M55-62.
149. Branen L, Hovgaard L, Nitulescu M et al. Inhibition of tumor necrosis factor-alpha reduces atherosclerosis in apolipoprotein E knockout mice. Arterioscler Thromb Vasc Biol 2004; 24:2137-2142.
150. Whitman SC, Ravisankar P, Daugherty A. IFN-gamma deficiency exerts gender-specific effects on atherogenesis in apolipoprotein E-/- mice. J Interferon Cytokine Res 2002; 22:661-670.
151. Legler DF, Micheau O, Doucey MA et al. Recruitment of TNF receptor 1 to lipid rafts is essential for TNFalphamediated NF-kappaB activation. Immunity 2003; 18:655-664.
152. Hunter I, Nixon GF. Spatial compartmentalization of tumor necrosis factor (TNF) receptor 1-dependent signaling pathways in human airway smooth muscle cells. Lipid rafts are essential for TNF-alpha-mediated activation of RhoA but dispensable for the activation of the NF-kappaB and MAPK pathways. J Biol Chem 2006; 281:34705-34715.
153. Higuchi M, Singh S, Jaffrezou JP et al. Acidic sphingomyelinase-generated ceramide is needed but not sufficient for TNFinduced apoptosis and nuclear factor-kappa B activation. J Immunol 1996; 157:297-304.
154. Gamard CJ, Dbaibo GS, Liu B et al. Selective involvement of ceramide in cytokine-induced apoptosis. Ceramide inhibits phorbol ester activation of nuclear factor kappaB. J Biol Chem 1997; 272:16474-16481.
155. Luberto C, Yoo DS, Suidan HS et al. Differential effects of sphingomyelin hydrolysis and resynthesis on the activation of NF-kappa B in normal and SV40-transformed human fibroblasts. J Biol Chem 2000; 275:14760-14766.
156. Hailemariam TK, Huan C, Liu J et al. Sphingomyelin synthase 2 deficiency attenuates NFkappaB activation. Arterioscler Thromb Vasc Biol 2008; 28:1519-1526.
157. Stone PH, Muller JE, Hartwell T et al. The effect of diabetes mellitus on prognosis and serial left ventricular function after acute myocardial infarction: contribution of both coronary disease and diastolic left ventricular dysfunction to the adverse prognosis. The MILIS Study Group. J Am Coll Cardiol 1989; 14:49-57.
158. Grynberg A, Demaison L. Fatty acid oxidation in the heart. J Cardiovasc Pharmacol 1996; 28 Suppl 1:S11-17.
159. Calvani M, Reda E, Arrigoni-Martelli E. Regulation by carnitine of myocardial fatty acid and carbohydrate metabolism under normal and pathological conditions. Basic Res Cardiol 2000; 95:75-83.
160. Augustus AS, Kako Y, Yagyu H et al. Routes of FA delivery to cardiac muscle: modulation of lipoprotein lipolysis alters uptake of TG-derived FA. Am J Physiol Endocrinol Metab 2003; 284:E331-339.
161. Jacob S. Lipid droplet accumulation in the heart during fasting. Acta Histochem 1987; 82:149-152.
162. Stanley WC, Lopaschuk GD, McCormack JG. Regulation of energy substrate metabolism in the diabetic heart. Cardiovasc Res 1997; 34:25-33.
163. Young ME, Guthrie PH, Razeghi P et al. Impaired long-chain fatty acid oxidation and contractile dysfunction in the obese Zucker rat heart. Diabetes 2002; 51:2587-2595.

164. Finck BN, Han X, Courtois M et al.. A critical role for PPARalpha-mediated lipotoxicity in the pathogenesis of diabetic cardiomyopathy: modulation by dietary fat content. Proc Natl Acad Sci USA 2003; 100:1226-1231.
165. Yokoyama M, Yagyu H, Hu Y et al. Apolipoprotein B production reduces lipotoxic cardiomyopathy: studies in heart-specific lipoprotein lipase transgenic mouse. J Biol Chem 2004; 279:4204-4211.