[Frontiers in Bioscience, Landmark, 22, 96-116, January 1, 2017]

Sphingolipids in obesity and related complications

Krishna M. Boini[1,2], Min Xia[1], Saisudha Koka[1], Todd W.B. Gehr[2], Pin-Lan Li[1]

[1]Department of Pharmacology and Toxicology, Virginia Commonwealth University, School of Medicine, Richmond, VA, 23298, USA, [2]Department of Nephrology, Virginia Commonwealth University, School of Medicine, Richmond, VA, 23298, USA

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Characteristics of obesity-associated sphingolipids
- *3.1. Ceramide*
- *3.2. Sphingomyelin*
- *3.3. Glycosphingolipids*
- *3.4. Sphingosine-1-phosphate*
- *3.5. Ceramide-1-phosphate*

4. Molecular mechanisms of sphingolipids in obesity
- *4.1. Membrane rafts*
- *4.2. Increase in caloric intake*
- *4.3. Fat storage disturbance*
- *4.4. Adipokine production*
- *4.5. Oxidative stress*
- *4.6. Inflammasome activation*
- *4.7. Lipotoxicity*

5. Pathophysiology of sphingolipids in obesity and related complications
- *5.1. Obesity-induced insulin resistance and diabetes mellitus*
- *5.2. Sphingolipids in hypertension*
- *5.3. Chronic kidney disease*
- *5.4. Atherosclerosis*
- *5.5. Endothelial dysfunction*

6. Therapeutic interventions by targeting sphingolipids
- *6.1. Inhibition of serine palmitoyl transferase (SPT) activity*
- *6.2. Inhibition of acid sphingomyelinase activity*
- *6.3. Targeting gangliosides*

7. Conclusion
8. Acknowledgements
9. References

1. ABSTRACT

Sphingolipids are biologically active lipids ubiquitously produced in all vertebrate cells. Aside from their role as structural components of cell membranes, sphingolipids also function as intracellular and extracellular mediators that regulate many important physiological cellular processes including cell survival, proliferation, apoptosis, differentiation, migration and immune processes. Recent studies have also indicated that disruption of sphingolipid metabolism is strongly associated with different diseases that exhibit diverse neurological and metabolic consequences. Here, we briefly summarize current evidence for understanding of sphingolipid pathways in obesity and its associated complications. The regulation of sphingolipids and their enzymes may have a great impact on the development of novel therapeutic modalities for a variety of metabolic diseases.

2. INTRODUCTION

The prevalence of obesity is growing at an alarming rate. This is of particular concern especially given that obesity is associated with an array of metabolic complications including chronic kidney disease, myocardial infarction, hypertension, atherosclerosis, insulin resistance and type 2 diabetes mellitus (1). In addition to the pathological effects in cardiovascular diseases and glucose metabolism, it is also closely

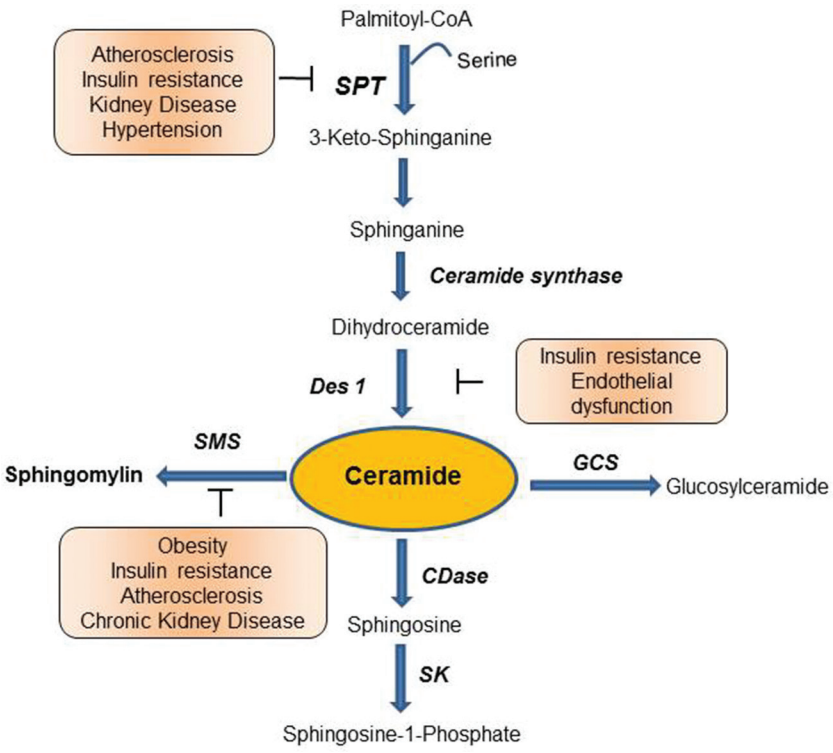

Figure 1. Sphingolipid biosynthetic pathways. Inhibition of indicated biosynthetic enzymes is associated with prevention of chronic metabolic diseases. SPT: Serine Palmotoyl transferase; DES1, Dihydroceramide saturase 1; SMS: Sphingomyelinase synthase; GCS: Glucosylceramide synthase; SK: Sphingosine kinase; Cdase: Cearmidase.

associated with dyslipidemia, evident by an increase in circulating lipids including triglycerides and free fatty acids (2). The dyslipidemia is thought to arise from an oversupply of both exogenous dietary chylomicron derived lipids along with endogenously synthesized lipids such as those released from the liver (i.e. cholesterol, triacylglycerol) and adipose tissue (i.e. free fatty acids). Traditionally, elevations of these commonly measured circulating lipids have been used clinically to assess the risk of metabolic and heart disease (3). While this approach has been useful, recently there has been an emergence of research attempting to identify other circulating lipid species that may prove to be more accurate biomarkers of disease onset and progression (4). In this regard, circulating sphingolipids have emerged as potential biomarkers and mediators of disease development (5, 6). In this review, we will summarize current knowledge regarding the functions of sphingolipids in obesity and related complications and provide novel insights into the pathways in common between sphingolipid metabolism and pathological features of these disorders.

3. CHARACTERISTICS OF OBESITY-ASSOCIATED SPHINGOLIPIDS

Sphingolipids are a major class of lipids that are ubiquitous constituents of eukaryotic membranes. They are composed of one polar head group and two nonpolar tails. At the core of all sphingolipids is the long-chain amino alcohol, sphingosine. Sphingolipids are synthesized in a pathway that begins in the endoplasmic reticulum and is completed in the Golgi apparatus. They are then transported via vesicles and monomeric transport to the plasma membrane and endosomes, where they perform many of their functions. On a broad scale, sphingolipids are typically classified as ceramides, sphingomyelins or glycosphingolipids (Figure 1). The addition of a long chain fatty acid, at the amine group of carbon 2 of sphingosine yields N-nacylsphingosine, commonly known as ceramide. Depending on the attachment of a mono- or oligosaccharide head group to the sphingosine base, different subclasses of glycosphingolipids are generated as described below. Detailed reviews of sphingolipid biosynthesis and metabolism have been previously published and will be reviewed only briefly here (7-9). These sphingolipids have been reported to be involved in the development of obesity and related complications (Table 1).

3.1. Ceramide

De novo ceramide synthesis begins in the endoplasmic reticulum with a condensation reaction between palmitoyl-CoA and serine leading to the formation of 3-Keto-dihydrosphingosine. This reaction

Table 1. A summary of effects on *in vivo* prevention of sphingolipid accumulation on obesity and associated complications

Metabolic condition	Rodent model	Treatment/mice	References
Atherosclerosis	ApoE-deficient mice	Myriocin	(48, 91)
Insulin resistance	HFD mice; Zucker fa/fa and dexamethasone-treated rats	Myriocin	(29)
Insulin resistance	Dexamenthasone-treated mice	DES1 -/+	(31)
Insulin resistance	Ob/ob mice	AMP-DNM	(59)
Insulin resistance	High fat fed mice	Genz-123346 GM3 -/-	(58-61)
Hypertension	HFD, SHRs, Wild type	Myriocin, Losartan	(66, 67)
Chronic kidney disease	HFD	Amitriptyline	(24)

is catalyzed by serine palmitoyltransferase (encoded by one of three *SPTLC* genes). 3-keto-dihydrosphingosine is then reduced to form sphinganine, which is acylated by a ceramide synthase to form dihydroceramide (10). Dihydroceramide is then converted into ceramide by dihydroceramide desaturase. Six individual ceramide synthases, LASS1-6, are known to catalyze the formation of dihydroceramides or ceramides (depending on whether the substrate is dihydrosphingosine or sphingosine, respectively). Recently, a more complex mechanism regulating cellular ceramide levels has been found to involve the salvage or recycling pathway.

In the salvage pathway, ceramide is hydrolyzed by ceramidases to sphingosine, which is then re-acylated via the action of ceramide synthases to regenerate ceramide. Ceramide is the fundamental structural unit common to all sphingolipids. However, in addition to forming the basis for sphingolipid and sphingomyelin biosynthesis, it is now known that ceramide can act as a signaling molecule in its own right, being involved in signal transduction, cellular differentiation and proliferation, as well as apoptosis and degeneration of cells. These topics have been reviewed extensively previously (11-13). One of the most important reproducible findings for ceramide is its ability to elicit apoptosis. Ceramide mediates apoptosis via several different downstream targets including death-associated protein kinase, kinase suppressor of Ras, protein kinase C, Rac, inducible nitric oxide synthase, ceramide-activated protein phosphatase, and c-Jun N-terminal kinase. At higher concentrations, ceramide has been shown to elicit its proapoptotic effects by increasing intracellular reactive oxygen species (ROS). In this context, ceramide and ROS have been associated with mitochondrial dysfunction and release of proapoptotic cytochrome C (14). Given the multitude of cellular effects elicited by ceramide, it is not surprising that diverse and sometimes contradictory effects of ceramide have been reported. These may have resulted from developmental, cell-type specific, compartment-specific or concentration-dependent effects of ceramide, or by unknown contribution of downstream sphingolipids. More complex sphingolipids are formed by addition of polar head groups at the 1-hydroxy position of ceramide. These include the sphingomyelins and glycosphingolipids (such as cerebrosides, sulfatides, globosides and gangliosides).

3.2. Sphingomyelin

Sphingomyelin, accounting for ~10% of mammalian cellular lipids, is the major representative of phosphosphingolipids. The sphingomyelins are synthesized by the transfer of phosphorylcholine from phosphatidylcholine to ceramide in a reaction catalyzed by sphingomyelin synthase. They are important constituents of the cell membrane and are particularly enriched in the myelin sheath. In the hydrolytic pathway, sphingomyelin is cleaved by one of several sphingomyelinases (SMases) (encoded by *SGMS1-4*), releasing phosphocholine and ceramide. These include lysosomal acid sphingomyelinases, zinc-dependent secretory sphingomyelinases, neutral magnesium-dependent sphingomyelinases, and alkaline sphingomyelinases, which can be distinguished according to their pH optima and subcellular localization. Defects in the enzyme that degrades sphingomyelin, acid sphingomyelin phosphodiesterase (*SMPD1*), result in the lysosomal storage diseases such as Niemann-Pick disease. Over 100 mutations in the *SMPD1* gene have been found to cause Niemann-Pick disease (15).

3.3. Glycosphingolipids

Glycosylation of ceramide forms a group of glycosphingolipids with diverse structures and a characteristic motif common to all glycosphingolipids, which is a monosaccharide, either glucose (the glucosylceramides) or galactose (galactosylceramides), bound directly to ceramide through a beta-glycosidic linkage. Glycosphingolipid composition varies depending on the cell type, developmental stage and aging (16).

3.3.1. Glucosylceramides

Glucosylceramide is generated from ceramide by the action of ceramide glucosyltransferase (encoded by the gene *UGCG*). Stepwise elongation gives rise to either the gangliosides or globosides. The gangliosides

have at least three sugars at the 1-hydroxy position, one of which must be sialic acid (a.k.a. n-acetylneuraminic acid). The 60+ known gangliosides differ mainly in the position and number of sialic acid residues. The specific names for gangliosides are a key to their structure. The letter G refers to ganglioside, and the subscripts M, D, T and Q indicate that the molecule contains mono-, di-, tri and quatra(tetra)- sialic acid. The numerical subscripts 1, 2 and 3 refer to the carbohydrate sequence that is attached to ceramide. For example, GM2 has a single sialic acid residue on the second carbohydrate attached to the ceramide backbone. Gangliosides are particularly enriched in neuronal membranes of the central nervous system, and errors in ganglioside metabolism and degradation often lead to severe neurological symptoms. Mutations in genes encoding enzymes of ganglioside metabolism cause lipid storage diseases called gangliosidoses, that comprise GM1 gangliosidosis and GM2 gangliosidosis (Tay-Sachs Disease and Sandhoff disease) (17).

Globosides represent cerebrosides that contain additional carbohydrates, predominantly galactose, glucose or N-acetylgalactosamine. Different globosides are found in various organs outside of the brain. For example, lactosyl ceramide is a globoside found in erythrocyte plasma membranes. Globotriaosylceramide (also called ceramide trihexoside) contains glucose and two molecules of galactose and it accumulates primarily in the kidneys of patients suffering from Fabry disease.

3.3.2. Galactosylceramides

Galactosylceramides are major constituents of oligodendrocyte cell membranes, which form myelin. Cerebrosides constitute the predominant species of galactosylceramide: the glucocerebrosides (with an additional glucose) or galactocerebrosides (containing an additional galactose). Galactocerebrosides are the most abundant type in cell membranes. In contrast, the glucocerebrosides represent intermediates in the synthesis or degradation of more complex glycosphingolipids and are not normally found in membranes. Excess lysosomal accumulation of the glucocerebrosides is observed in Gaucher's disease (17). Addition of a sulfate group to a cerebroside yields a sulfatide. Sulfatides are the second major lipid component of oligodendrocyte membranes. Together, these two glycolipids comprise 27% of total myelin lipid (17). An accumulation of sulfatides is observed in the lipid storage disorder, metachromatic leukodystrophy (18).

3.4. Sphingosine-1-phosphate

Sphingosine is the major free sphingoid base in mammalian cells and is produced solely through ceramidases. Once generated by the ceramidase, sphingosine can be reacylated to reform ceramide as a part of the "salvage" pathway or phosphorylated by sphingosine kinase to form sphingosine-1-phosphate (S1P). Free sphinganine is the less abundant, saturated counterpart of sphingosine. Sphinganine is produced either through the de novo pathway or by the action of SMases and ceramidases on sphinganine-containing substrates and it is an alternative substrate for the sphingosine kinases.

S1P is a product of sphingosine kinase, which can act intracellularly to regulate cell function and gene expression. S1P can also be secreted, exerting extracellular effects as an autocrine/paracrine mediator. Evidence from C2C12 myotubes also suggests that S1P can be generated at the extracellular leaflet of the plasma membrane by sphingosine kinase and can act in an autocrine manner (19). Five G protein-coupled surface receptors, termed S1P1–5 (formerly known as Edg1, Edg5, Edg3, Edg6, and Edg8, respectively), are activated by S1P, diversifying the biological effects of this lipid (20).

3.5. Ceramide-1-phosphate

Although ceramide is primarily converted into more complex sphingolipids in the Golgi, ceramide can also be phosphorylated to produce ceramide-1-phosphate (C1P). C1P is produced in the trans-Golgi and potentially the plasma membrane by ceramide kinase (CERK). CERK, a member of the diacylglycerol kinase family, was originally identified based on its homology to sphingosine kinase. Unlike sphingosine kinases, CERK only utilizes ceramide as a substrate and has no activity for sphingosine or diacylglycerol. CERK activity is enhanced in the presence of calcium or magnesium and contains a putative calmodulin-like domain. In addition, ceramide kinase has specificity for sphingosine-containing ceramides since it has very low activity against dihydroceramide and phytoceramide species (21). Among the ceramide species it recognizes, CERK prefers ceramide species with acyl chain lengths greater than 12 carbons long; however, no preference was observed for the degree of saturation (21).

4. MOLECULAR MECHANISMS OF SPHINGOLIPIDS IN OBESITY

4.1. Membrane rafts

Membrane rafts (MRs) have been implicated in the development of obesity and related complications such as chronic kidney disease, type 2 diabetes and endothelial dysfunction. Accumulating evidence has demonstrated that MRs or caveolae are present in various target tissues of insulin resistance such as striated muscle, adipose tissue, the liver, and pancreatic β cells that secrete insulin (22). Although the role of MRs in mediating insulin signaling is controversial, it is well accepted that MR-dependent interactions may help segregate signaling components because raft perturbation changes the sensitivity of two key insulin receptor-mediated signaling pathways, activating the small guanosine

triphosphatase TC10 and phosphoinositide 3-kinase. MRs are clearly important to insulin signaling and may thereby determine the insulin resistance during obesity or diabetes. Another line of evidence corroborating the involvement of MRs in obesity is related to observations in Obese Zucker fa/fa rats and ob/ob mice with increased levels of GM3 synthase mRNA in their adipose tissues. Addition of GM3 to 3T3-LI adipocytes suppresses insulin-stimulated phosphorylation of the insulin receptor, suggesting that MRs containing GM3 are involved in the signaling process of the insulin receptors. Indeed, other studies have shown that insulin signaling is initiated in glycosphingolipid-enriched rafts and caveolae (23).

With respect to the role of MR redox signaling platforms in the development of obesity, some preliminary experiments were recently performed in our laboratory to test whether excessive accumulation of sphingolipids, ceramide, and their metabolites, or a combination contributes to the development of obesity and associated organ damages. In these experiments, a high-fat diet (HFD) significantly increased plasma total ceramide levels compared with animals fed a low-fat diet (LFD). Treatment of mice with the ASMase inhibitor amitriptyline significantly attenuated the HFD-induced plasma ceramide levels. Correspondingly, HFD-induced increases in body weight, plasma leptin concentration, urinary total protein and albumin excretion, glomerular damage indexes, and adipose tissue ASMase activities were almost completely suppressed. HFD-induced reduction of insulin receptor sensitivities were also blocked by ASMase inhibition. These results provided evidence that ceramide biosynthesis plays a pivotal role in the development of obesity, metabolic syndrome, and associated kidney damages (24).

MR redox signaling platforms were also shown to be associated with obesity. Using glomerular capillary endothelial cells (GECs), visfatin, an adipokine was found to stimulate ASMase activity and led to aggregation of ceramide with NADPH oxidase subunits, $gp91^{phox}$ and $p47^{phox}$, a typical MR redox signaling platform, where $O_2^{\bullet-}$ production increased. The ASMase inhibitor, amitriptyline, or ASMase siRNA blocked this visfatin-induced formation of MR redox signaling platforms associated with NADPH oxidase and $O_2^{\bullet-}$ production. The results suggest that the injurious effect of visfatin, is associated with the formation of MR redox signaling platforms via MR clustering, where $O_2^{\bullet-}$ production increases the glomerular permeability by disruption of microtubule networks in GECs leading to glomerular injury (25).

4.2. Increase in caloric intake

Obesity is a condition in which body weight, caused by excessive accumulation of stored body fat, is increased to the point where it becomes a risk factor for certain health conditions and mortality. Overweight and obese individuals are at an increased risk for hypertension, dyslipidemia, type 2 diabetes, heart disease, stroke and certain forms of cancer. Unfortunately, obesity is growing worldwide epidemic with over 1 billion of the global population either overweight or clinically obese. It is well-known that a high calorie diet rich in saturated fats contributes to excessive weight gain. However, the role that saturated fats play in this process goes far beyond simple storage in fat tissue. Saturated fats are essential building blocks for the bioactive lipid ceramide. Accumulation of ceramide has recently been associated with obesity. However, it is not known whether its accumulation plays an active role in the induction of obesity. In this regard, Walls *et al.*, utilized genetic manipulation in *Drosophila* to accumulate and deplete a variety of ceramide species and other related lipids. Using this approach, the authors showed that modulation of ceramide and related lipids is sufficient to induce obesity through two distinct mechanisms: a caloric intake-dependent mechanism that works through suppression of neuropeptide Y satiety signaling, and a caloric intake-independent mechanism working through regulation of hormone producing cells that regulate fat storage (26). These data implicate ceramides in actively promoting obesity by increasing caloric intake and fat storage mechanisms.

4.3. Fat storage disturbance

Adipose tissue is remarkably flexible in terms of energy storage and release. Responding to hormonal and energetic cues, it serves as a source of energy-rich fatty acids during times of negative energy balance, reducing its lipid store and releasing fatty acids to target tissues in need of energy. In contrast, adipocyte lipid uptake, esterification, and storage in the form of triglyceride within the lipid dropl *et al* lows for expansion of adipose tissue, a beneficial, adaptive response to over nutrition that can prevent ectopic lipid deposition and lipotoxicity in other cell types. Triglyceride stored within the lipid droplet is hydrolyzed to fatty acids and released to fuel peripheral tissues upon metabolic demand.

Obesity increases lipid accumulation in non-adipose tissues (27, 28). The saturated fat storage capacity of adipose tissue spills free fatty acids (FFAs) into the circulation with lipolysis and leads to accumulation of ectopic fat in tissues not suited for fat storage. Increased FFAs and cytokines activate immune receptors and stress signaling pathways that interfere with insulin signaling in muscle and liver (29). As a non-oxidative pathway of FFAs, intracellular and circulating ceramide are elevated and bioactive sphingolipids such as ceramide, sphingosine, and sphingosine 1-phosphate (S1P) are now known to link overnutrition, inflammation, and metabolic dysregulation. Accumulating evidence suggests that ceramide synthesis can be activated by increased availability of free fatty acids, proinflammatory cytokines, oxidative stress, and hormones (5, 30). All of

these conditions represent the obese conditions of adipose tissue and suggest that ceramide metabolism may be altered in obesity. Indeed, ceramide levels were elevated in skeletal muscle, liver, and hypothalamus of obese rodents and human (29). Samad *et al.* (5) demonstrated that total sphingomyelin and ceramide levels were reduced in the adipose tissues from leptin-deficient ob/ob mice. In contrast, plasma sphingomyelin, ceramide, sphingosine, and S1P were elevated in plasma. Since expression of ceramide synthetic genes including SPT, neutral SMase, and ASMase is upregulated in adipose tissue, these opposite sphingolipid profiles in plasma and adipose tissue suggest that secretion of ceramide from adipose tissues into circulation is increased (5).

4.4. Adipokine production

Adipokines have been suggested as useful biomarkers for cardiovascular disease and metabolic dysregulation associated with obesity. Depending on the fat contents of the body, the types and amounts of adipokines secreted from adipose tissues vary. Some adipokines have beneficial effects by regulation of body weight due to reduced food intake/energy expenditure and reduction of inflammation and insulin sensititivity. Among these adipokines, leptin is one of the most active regulators of body weight and food intake. Dysregulation of leptin usually results in obesity and eventually insulin resistance and type 2 diabetes (31, 32). Rats treated with leptin display systemic improvements in insulin sensitivity and have suppressed de novo ceramide synthesis (33). More recently, Bonzon-Kulichenko *et al.* have demonstrated that central leptin infusion into the hypothalamus of mice reduces total ceramide content in white adipose tissue (WAT) via the action through the autonomic nervous system (34). Central leptin represses serine palmitoyl transferase (SPT), the rate-limiting enzyme for ceramide production in WAT, by 30%. Furthermore, the reduction of ceramides in WAT coincides with improved systemic insulin sensitivity. These results suggest that reduction of WAT ceramides contributes to improvements in systemic insulin sensitivity (34). The other adipokine, adiponectin, exerts its beneficial metabolic effects by lowering cellular ceramide levels. Furthermore, adiponectin is potently anti-apoptotic in the context of cardiomyocytes and pancreatic β cells *in vivo* through the lowering of cellular ceramides. The adiponectin receptors, AdipoR1 and AdipoR2 exhibit ceramidase activity in an adiponectin-dependent manner (35). Whether the receptors themselves contain the ceramidase activity or they sequester or induce a ceramidase upon activation is still unclear.

Another adipokine visfatin has also been found to be mainly expressed in visceral adipose tissue despite some controversial reports at the beginning (36). Recently, visfatin has been identified as a major injurious factor during obesity-associated diseases including diabetes, carotid and coronary atherosclerosis, and chronic kidney disease (25). It has been postulated that visfatin may play a role in innate immunity during inflammation and obesity (a low-grade inflammatory process). Visfatin expression and plasma levels of visfatin are associated with obesity in animals and humans (25). Furthermore, the plasma levels of visfatin were significantly increased in HFD-fed mice compared to normal chow-fed mice. Recent studies have also reported that the plasma visfatin level was significantly increased from 29 ng/mL in normal controls to 41 ng/mL in a large population of patients with chronic kidney disease (CKD) (25). In contrast to adiponectin, the higher the plasma level of visfatin, the more severe the CKD, and a higher plasma visfatin level predicts a higher mortality rate in patients of CKD. Most recently, we found that visfatin induces membrane raft clustering associated with ceramide accumulation in endothelial cells to form the redox signaling platform on the cell membrane, which mediates NADPH oxidase activation, resulting in increased $O_2^{\bullet-}$ production and endothelial dysfunction (37). These observations highlight the dual role of adipokines in sphingolipid metabolism during obesity.

4.5. Oxidative stress

Products of sphingolipid metabolism are associated with oxidative stress. Also, stimuli of ceramide production, for example, obesity, diabetes, tissue necrosis factor, chemotherapeutic agents, and radiation increase oxidant production. These associations suggest that sphingolipids may act as second messengers to increase oxidant production. A large number of studies have been conducted over the past decade to test this. However, the cellular mechanism remains elusive. There appears to be a complex, two-way interaction between sphingolipids and oxidant production. Increased ROS activity, decreased in antioxidant defenses, and activated nitric oxide synthase or NADPH oxidase can stimulate turnover of complex sphingolipids and generation of bioactive sphingolipid metabolites, for example, ceramide, sphingosine, and sphingosine-1- phosphate. Conversely, ceramide analogs act directly on isolated mitochondria to inhibit mitochondrial electron transport at complex III, increasing ROS production. Some agents that inhibit complex III (e.g., TNF, adriamycin, tamoxifen) may do so by increasing mitochondrial ceramide levels (38). The interplay between sphingolipid signaling and oxidant activity likely contributes to the onset and propagation of oxidative stress in obesity and its associated complications such as inflamation, diabetes, atherosclerosis, chronic kidney diseases and hypertension.

4.6. Inflammasome activation

Accumulating evidence demonstrated that the sphingolipid, ceramide and ASMase initiate inflammasome formation and activation in obesity and different pathological conditions. Knockouts of genes encoding

NLRP3 inflammasome molecules (such as NLRP3$^{-/-}$, ASC$^{-/-}$, and caspase-1$^{-/-}$) significantly protected mice from HFD-induced obesity, increased adiposity, insulin resistance, glucose intolerance, and inflammation (39) The expression of the NLRP3 inflammasome subunits in adipose tissue correlates directly with body weight in mouse models and obese individuals with type 2 diabetes mellitus (40). In our recent studies, mice lacking the ASC gene demonstrated significant attenuation of HFD-induced obesity compared with ASC$^{+/+}$ mice, and they were also protected from obesity-associated glomerular and podocyte injury (41). The mechanism of HFD-induced inflammasome activation may be due to the high production of the fatty acid metabolites including ceramide and palmitate, as they have been shown to induce inflammasome activation through a mechanism that involves defective autophagy and accumulation of mtROS. In this regard, Vandanmagsar *et al.* showed that adding ceramide to adipose tissue explants led to NLRP3-dependent IL-1β production, suggesting that ceramide acts a danger signal to stimulate the NLRP3 inflammasome activation (40). Moreover, we recently found that ASM gene knockout mice significantly attenuates the ceramide production, NLRP3 inflammasome formation and activation in glomeruli and protects against HFD-induced glomerular injury (Unpublished observations). Hence, more studies are imperative to explore whether abnormal activation of NLRP3 inflammasomes senses ceramide in adipose tissue of obese individuals to reveal new therapeutic targets for the prevention or treatment of obesity and related pathologies.

4.7. Lipotoxicity

Lipotoxicity is a consequence of delivering lipids to non-adipose tissues in excess of their oxidative or storage capacities, causing cell dysfunction or death. Although fatty acids are widely recognized as mediators of lipotoxicity (42), roles for sphingolipids and ceramides have also been suggested (38). Ceramides comprise a family of lipid signaling molecules generated from fatty acids and sphingosine (38). Long-chain ceramides of 16 to 24 carbons in length naturally occur and are distributed in cell membranes, and they have both structural and functional roles for mammalian cells. These molecules influence signaling pathways that regulate cell growth, proliferation, motility, adhesion and differentiation (38). In contrast, ceramides with shorter carbon chains (two to six carbons) promote cell senescence, cytotoxicity, apoptosis, insulin resistance and inflammation, and inhibit survival and growth (38).

Disease-associated lipolysis, a feature of insulin resistance, is initiated by critical levels of stress in the endoplasmic reticulum and mitochondrial dysfunction (43). In such states, ceramides cause insulin resistance by activating proinflammatory cytokines and inhibiting insulin-stimulated signaling through PI3K-Akt (44). In obesity, adipose tissue, skeletal muscle and the liver exhibit abnormalities in sphingolipid metabolism that result in increased ceramide production, inflammation and activation of proinflammatory cytokines. These abnormalities are also involved in impairments of glucose homeostasis and insulin responsiveness (38). In C57BL/6J wild type mice with diet-induced obesity and type 2 diabetes mellitus (45), ceramide levels in adipose tissue are elevated; the mechanism likely involves increased activation of sphingomyelin transferase and acidic and neutral sphingomyelinases (46).

Another consequence of obesity is the increased risk of atherosclerosis, initiated by the aggregation of low density lipoprotein (LDL) within the arterial wall. Exciting new studies have demonstrated that ceramide or other sphingolipids may play a critical role in the progression of the disease. First, the inclusion of excess sphingolipids in the diet of LDL receptor knockout mice significantly influences the formation of atherosclerotic lesions (47). Second, treating apolipoprotein E knockout mice, which are susceptible to atherosclerosis, with the SPT inhibitor myriocin dramatically, reduces lesion area formation (48). Several mechanisms have been proposed by which ceramide may contribute to the formation of atherosclerotic lesions. Due to its hydrophobicity and capacity to undergo extensive hydrogen bonding, ceramides have a pronounced tendency for self-aggregation (49). Thus, several groups have proposed that ceramide generation in LDL contributes to aggregation of LDL which drives formation of atherosclerotic plaques. Support for this hypothesis includes the following: the ceramide content of aggregated LDL is 10–50 fold higher than that of plasma LDL (50) and exposing LDL particles to a bacterial sphingomyelinase promotes LDL aggregation (50). Alternatively, sphingomyelin has been proposed to block the uptake of LDL by blocking access to apolipoprotein E and lipoprotein lipase (51). Moreover, ceramide has been shown to induce apoptosis of certain cells lining the vascular wall, a process implicated in plaque erosion and thrombosis. Lastly, the ceramide metabolite, S1P, stimulates the proliferation of endothelial and smooth muscle cells in vascular walls (52), which would promote thickening and favor plaque stabilization.

5. PATHOPHYSIOLOGY OF SPHINGOLIPIDS IN OBESITY AND RELATED COMPLICATIONS

Sphingolipids were initially thought to represent structural membrane components only; however, emerging evidences now reveal additional biological and functional roles for this class of lipids, including cellular recognition and adhesion, signal transduction, growth regulation, and differentiation, among others. Because sphingolipids have been associated with a diversity of biological processes, a dysfunction in the synthesis and/or dysregulation of sphingolipids would be expected to have a major impact on metabolic diseases. Within the past decade, progress has been made in our understanding

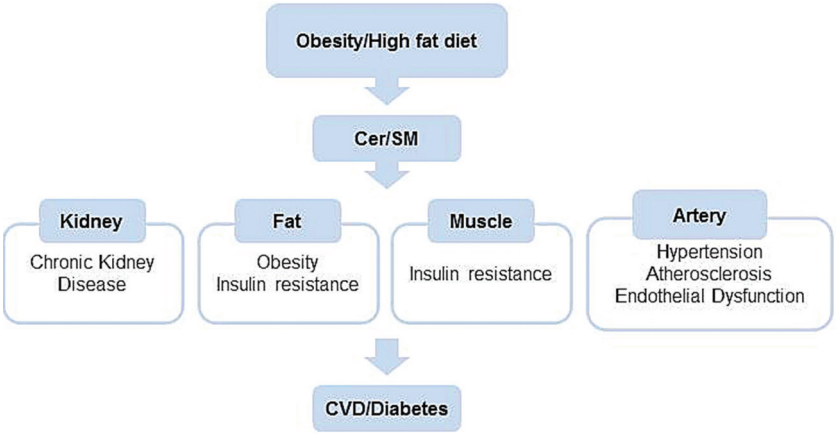

Figure 2. Elevated ceramide and sphiomyelin in Obese adipocytes elicit the pathophysiological events in various tisses and organs. CVD: Cardiovascular disease.

of how sphingolipids contribute to disease processes, which is leading to novel therapeutic approaches based on interventions in sphingolipid homeostasis. We will now summarize some of the areas in which particularly important advances have been made regarding obesity and associated complications (Figure 2).

5.1. Obesity-induced insulin resistance and diabetes mellitus

Many studies have confirmed the contribution of sphingolipids to the pathogenesis of diabetes mellitus. Recently, we also tested the role of sphingolipids in the development of obesity, and some studies were performed to test whether excessive accumulation of sphingolipids, ceramide, and their metabolites, or a combination contributes to the development of obesity and associated organ damages. In these studies, HFD significantly increased plasma total ceramide levels compared with animals fed a LFD. Treatment of mice with the ASMase inhibitor amitriptyline significantly attenuated the HFD-induced plasma ceramide levels. Correspondingly, HFD-induced increases in the body weight gain, plasma leptin concentration and adipose tissue ASMase activities were almost completely suppressed. HFD-induced reduction of insulin receptor sensitivities were also blocked by ASMase inhibition. These results provided evidence that ceramide biosynthesis may play a pivotal role in the development of obesity and associated insulin resistance or diabetes (24).

In addition, obese leptin deficient ob/ob mice, Zucker fa/fa rats, ZDF rats, db/db mice and HFD-fed animals display evidence of increased obesity, inflammation and dyslipidema. Treating ZDF and Zucker fa/fa rats with the SPT inhibitor myriocin prevented aberrant ceramide accumulation in muscle, liver, and serum and improved glucose tolerance and insulin sensitivity (29). Similarly, oral doses of myriocin in HFD-induced obese mice displayed improvements in insulin sensitivity, as measured by circulating insulin levels during glucose tolerance tests (29). In fact, the improvement in insulin sensitivity by myriocin was similar to that with rosiglitazone, one of the most effective insulin-sensitizing drugs currently marketed. Fenretinide, a chemotherapeutic agent that lowers circulating retinol-binding protein levels, improves insulin sensitivity in high fat diet-fed mice (53). This drug was recently identified as an inhibitor of dihydroceramide desaturase (DES1) (31); thus, some of its insulin-sensitizing actions may result from effects on ceramide metabolism.

The clinical studies also showed the significant elevation of plasma ceramide levels in patients with obesity and diabetes mellitus (54, 55). The Roux-en-Y bypass gastric surgery significantly decreased the plasma ceramide level in severely obese patients. After 6 months post operation, the reduction of the total ceramide level was also significantly correlated with excessive weight loss, improvement in insulin sensitivity and decreases in TNFα concentration (56). The authors suggest that a reduced inflammatory environment resulting from the loss of adipose tissue causing lipotoxicity and cellular dysfunction may explain the observed improvement in insulin sensitivity. Incubation of human pancreatic β cells with S1P receptor agonist did not have a negative influence on the insulin secretion triggered by glucose administration and did not induce apoptosis (57).

Studies with GM3 synthase null mice and inhibitors of glucosylceramide synthase suggest that gangliosides may also contribute to obesity-induced insulin resistance. Mice lacking the GM3 synthase gene display lower fasting glucose levels and improved glucose tolerance (58). When challenged with HFDs, the GM3 synthase null mice maintained improved glucose tolerance, insulin stimulated glucose uptake, and enhanced suppression of hepatic glucose output

measured by hyperinsulinemic-euglycemic clamps. The enhanced glucose homeostasis of GM3 synthase null mice strongly suggests that targeted pharmacological disruption of glucosylceramide-producing enzymes may provide an effective means of combating insulin resistance and type 2 diabetes. These reports were further confirmed in two studies. Using highly specific inhibitors of glucosyl ceramide synthase (GCS), N-(5-adamantane-1-yl-methoxy)-pentyl-1-deoxynojirimycin (AMP-DNM), Aerts *et al.* (59) demonstrated the ability to selectively decrease glucosylceramide content in muscle and liver of ob/ob mice without affecting ceramide content. Administration of the drug decreased fed blood glucose and improved glucose tolerance in ob/ob mice. Moreover, AMP-DNM increased whole body glucose clearance, while decreasing hepatic glucose output, under hyper-insulinemic conditions. Similar improvements were detected in diet-induced obese mice because fasting glucose and insulin were decreased in mice treated with the GCS inhibitor. Another study demonstrated that Genz-123346, a GCS inhibitor derived from PDMP doesn't stimulate ceramide accrual like the parent compound (60), but improves glucose homeostasis and insulin sensitivity in ZDF rats and HFD-fed mice (61).

The mechanistic studies were performed over the past decade have identified two distinct mechanisms that may be involved. Free fatty acids are going through an oxidative pathway to supply the energy for cell metabolism. Another route for FFAs is the sphingolipid biosynthetic pathway. Since FFAs are the substrate and major constituents for sphingolipids, ceramide is elevated in patients with obesity or diabetes and has a positive correlation with severity of insulin resistance (62). Accumulating evidence suggests that sphingolipid metabolism is a converging point linking excess FFAs and inflammation aroused by adipose-derived inflammation and that it contributes to progression of insulin resistance. Ceramide and sphingosine inhibit insulin actions and signaling by dephosphorylation and inhibition of AKT and AMPK activity in various cell culture systems (63). Holland *et al* (29) demonstrated that *in vivo* administration of myriocin, a specific SPT inhibitor, improved glucocorticoid, saturated fat and obesity-induced insulin resistance by inhibiting de novo ceramide synthesis.

Heterozygous deficiency of DES1 had improved insulin sensitivity and dexamethasone-induced insulin resistance was prevented (29). In cultured cells, the mechanisms of ceramide-mediated inhibition of insulin response were also confirmed. It has been demonstrated that ceramide antagonized dphosphorylation and activation of AKT and tyrosine phosphorylation of insulin receptor substrate (IRS-1) in 3T3-L1 adipocytes and C2C12 myocytes (64). Ceramide exerts its inhibitory effects by activating protein phosphatase 2A responsible for dephosphorylation of AKT. Additionally, ceramide activates PKCz and inhibits translocation of AKT to the membrane (65). By modulating AKT activity, ceramide inhibits insulin signaling pathway and ultimately the insulin response is altered.

5.2. Sphingolipids in hypertension

Sphingolipids may play a pathological role in hypertension. Noting the vascular effects of sphingolipids, Zhang *et al.* (66) sought to determine the role of sphingolipids in hypertension. They found that pharmacological inhibition of de novo ceramide synthesis using the SPT inhibitor myriocin and heterozygous deletion of DES1 prevented vascular dysfunction and hypertension in mice after HFD feeding. The authors also tested whether endothelial improvements were secondary to reduced vascular ceramide accrual or to improved systemic metabolism. They found that endothelial dysfunction, hypertension and endothelial nitiric oxide synthase phosphorylation evoked by HFD feeding are mediated to a significant degree by ceramide accumulation in the vasculature (66). In addition, Spijkers *et al.*, (67) showed that hypertension is associated with altered sphingolipid levels in both hypertensive humans and spontaneously hypertensive rats (SHRs). The vascular and plasma ceramide levels were higher in SHRs than in normotensive rats. Furthermore, in hypertensive patients, plasma ceramide levels correlated positively with their blood pressure (67). In *in vitro* experiments performed on carotid arteries isolated from SHRs, significant contraction of vessels was demonstrated after the application of SMase or sphingosine kinase inhibitors. These effects were not observed in arteries isolated from normotensive WKY rats (68). Presumably, ceramide, which causes elevated thromboxane A2 release from the endothelium, contributes to the contraction of the isolated arteries.

Additionally, *in vivo* administration of a sphingosine kinase inhibitor resulted in a marked rise in blood pressure in SHRs. Furthermore, these hypertensive rats have significantly increased levels of total ceramides in arterial tissues. Similaryly, Fryer *et al.* (69) observed dose-dependent and sustained elevation in mean arterial blood pressure in Sprague Dawley rats resulting from oral administration of a nonselective S1P receptor agonist (FTY720). Four weeks of treatment with losartan or hydralazine significantly decreased blood pressure and the vascular ceramide level, without a reduction of plasma ceramide concentration, suggesting that only vascular ceramide level is sensitive to antihypertensive therapy (67). The exact mechanism by which falling blood pressure leads to the reduction of vascular ceramide levels is currently unknown. Although stimulation of angiotensin II receptor type 2 increases cellular ceramide levels, it does not explain the interaction between losartan, an antagonist of angiotensin II type 1 receptor, and vascular levels of ceramide. A mechanism, which may explain the observed effect of both hydralazine

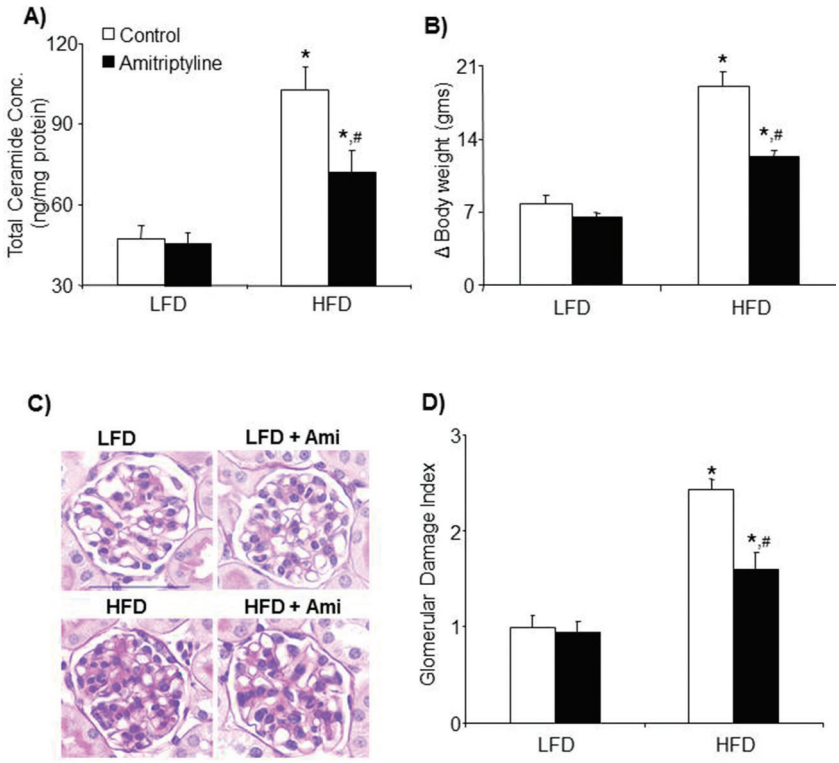

Figure 3. Plasma total ceramide concentrations, delta body weight, glomerular injury in C57BL/6J mice on low fat or high fat diet with or without amitriptyline treatment. Data are arithmetic means ± SE (n=4-12 each group) of plasma total ceramide concentrations (A), delta body weight (B), Photomicrographs show typical glomerular structure (original magnification, x400) in LFD or HFD treatment with or without amitriptyline treatment (C) Summarized data of glomerular damage index (GDI) by semi-quantitation of scores in 4 different groups of mice (n=6 each group). For each kidney section, 50 glomeruli were randomly chosen for the calculation of GDI in LFD or HFD fed C57BL/6J mice with or without amitriptyline treatment (D). *Significant difference (P<0.0.5) compared to the values from mice receiving the LFD, # Significant difference (P<0.0.5) compared to the values from mice receiving the HFD. Scale bar represents 50 μm.

and losartan on the vascular ceramide level, has been proposed by Czarny *et al.* The authors suggested that neutral SMase in plasma membrane acts as a mechano sensor, whose activity may be enhanced by high shear stress, which results in the generation of ceramide (70). In addition, Fenger *et al.* (71) in a genomic analysis demonstrated that the ceramide/S1P rheostat has a substantial influence on blood pressure regulation. Interestingly, they found that genes involved in de novo ceramide synthesis, rather than ceramide formation via SMase, were the most important sources of ceramide in a hypertensive population. The link between hypertension and ceramides suggests a novel pathophysiological mechanism leading to endothelial dysfunction and abnormal blood pressure regulation, which needs to be determined. This mechanism can also suggest a target for new drugs modulating the sphingolipid system and metabolism to improve the pharmacological treatment of hypertension.

5.3. Sphingolipids in chronic kidney diseases

Recent studies have indicated that ceramide might be implicated in the regulation of kidney function (72-75) in different pathological conditions such as obesity, hyperhomocysteinemia or diabetes (72-75). More recently, our group demonstrated that ceramide importantly contributes to the development of chronic glomerular injury associated with obesity, and therefore ceramide may serve as an important mechanism of end-stage renal disease (24). Several studies that employed thin layer chromatography and high performance liquid chromatography analysis reported the detection of ceramide in the kidney, leading to the hypothesis that ceramide may be involved already in the regulation of normal renal function (72-75). To determine whether ceramide also participates in the development of chronic renal failure, we employed a model of obesity-induced renal injury. We found that plasma ceramide levels were significantly increased in wild type mice fed a HFD compared to normal diet fed mice. Correspondingly, HFD-fed mice had significantly increased urinary total protein and albumin excretion, and glomerular damage index compared with normal diet fed mice. However, treatment with the acid sphingomyelinase inhibitor, amitriptyline, significantly decreased the HFD-induced ceramide production and attenuated the HFD-induced urinary total protein, albumin excretion, glomerular injury and podocyte injury (Figure 3). These data provide direct evidence that

the ceramide pathway is critically involved in obesity-induced glomerular injury and glomerular sclerosis, and therefore this sphingomyelinase pathway could be a target of therapeutic strategy for obesity and related end-stage renal damage (24). Further mechanistic studies have demonstrated that visfatin, a novel adipokine and injurious factor during obesity-induced chronic kidney disease stimulated ceramide production in glomerular capillary endothelial cells (GECs) (25) and that ceramide appears to be an important regulator of the function of glomerular filtration membrane, which is consistent with earlier observations that ceramide may be involved in the regulation of normal renal function (72, 73). It was also found that visfatin stimulation increased ASMase activity and led to aggregation of ceramide with NADPH oxidase subunits, $gp91^{phox}$ and $p47^{phox}$, a typical MR redox signaling platform, where $O_2^{\bullet-}$ production is increased in GECs. The ASMase inhibitor, amitriptyline, or ASMase siRNA blocked this visfatin-induced formation of membrane raft redox signaling platforms associated with NADPH oxidase and $O_2^{\bullet-}$ production. These results suggest that the injurious effect of the adpokine, visfatin, is associated with the formation of MR redox signaling platforms via MR clustering, where $O_2^{\bullet-}$ production increases the glomerular permeability by disruption of microtubule networks in GECs leading to glomerular injuries (25).

Additional studies performed on mesangial cells isolated from the kidney of obese diabetic rats have proven that S1P-induced mesangial cell proliferation both under normoglycemic and hyperglycemic conditions and it enhanced the expression of fibronectin (76). In clinical studies, ceramides also play a regulatory role in pathways, which may lead to a loss of renal function. Many investigations suggest that ceramides are also involved strongly in the pathogenesis of insulin resistance, which together with other metabolic syndrome elements are known to be risk factors of diabetes complications and macrovascular disease in patients with and without obesity and type 2 diabetes (57). The mechanism altering kidney cell membranes ceramide composition and association between plasma lipoproteins and kidney cell sphingolipid metabolism remains unclear, and the pathophysiological implications of ceramide changes in cell membrane or as signaling molecule in the development of obesity or diabetes mellitus and associated albuminuria or glomerular injury are yet to be determined (77).

5.4. Sphingolipids in atherosclerosis

Obesity-induced inflammation is implicated in increased risk of coronary artery disease and atherosclerosis. Atherosclerosis is an inflammatory disease characterized by increased production of a wide range of chemokines and cytokines. Early stage of atherogenesis involves the interaction of cholesterol-rich lipoproteins with the arterial wall (78). The processes implicated in early atherogenesis include lipoprotein oxidation (79, 80), lipoprotein retention and aggregation (57), endothelial alteration, monocyte recruitment, macrophage chemotaxis and foam cell formation, and smooth muscle cell migration and alteration (78). Several early reports demonstrated the importance of sphingomyelin in atherogenesis, which demonstrated that sphingomyelin accumulates in atherosclerotic plaques formed in human and animal models (81-83) and that LDL extracted from human atherosclerotic lesions has higher sphingomyelin levels than LDL from plasma (50, 84). A substantial amount of sphingomyelin found in the arteries and atherosclerotic lesions appears to arise from sphingomyelin synthesis in arterial tissues (85, 86). Plasma sphingomyelin levels in atherogenic apoE KO mice are four folds higher than in wild type mice (87), and this may contribute to the increased atherosclerosis (88, 89). Clinically, Jiang *et al.* also found that human plasma sphingomyelin levels and sphingomyelin/ phosphatidylcholine (PC) ratio are independent risk factors for occurrence of coronary heart disease (50, 90).

Park *et al.* (91) also reported that apoE knockout mice fed with a HFD, have elevated circulating sphingolipids (48). However, treatment with the de novo ceramide synthesis inhibitor, myriocin, not only reduced plasma ceramide and sphingomyelin, but also total plasma cholesterol and triglycerides. Further, inhibition of ceramide synthesis in the apoE KO mice resulted in a substantial reduction in atherosclerotic lesion area in the aortic root and slowed the progression of atherosclerosis in the brachiocephalic artery. Macrophage content in the aortic root was similarly reduced in myriocin-treated mice (48). Interestingly, inhibition of de novo ceramide synthesis also results in increased hepatic apoA-I synthesis and elevated circulating levels of favorable high density lipoprotein cholesterol (92). Similar to treatments using inhibitors of ceramide synthesis, the use of a myriocin-based S1P homologue, FTY720 also inhibits atherosclerosis (93).

5.5. Sphingolipids in endothelial dysfunction

Endothelial dysfunction derived from obesity may be mediated by lipotoxic metabolites. A growing body of literature suggests that nitric oxide (NO) is a major modulator to maintain vascular function (94, 95). As a ubiquitous signaling molecule, endothelial NO is responsible for regulation of vasodilation (96). Imbalance between production and degradation of NO may lead to occurrence of cardiac events. Obesity mediates increased plasma free fatty acids and increased ceramide contents in various tissues contributing to cardiovascular complications. Especially, ceramide inhibits signaling kinases that phosphorylate endothelial NO synthase (eNOS) at positive regulation site and activates signaling kinases that phosphorylate eNOS at negative regulatory sites (97, 98). Recently, Zhang *et al* (66) reported that inhibition of de novo ceramide biosynthesis by myriocin ameliorates the blood pressure accrual in mice fed a HFD.

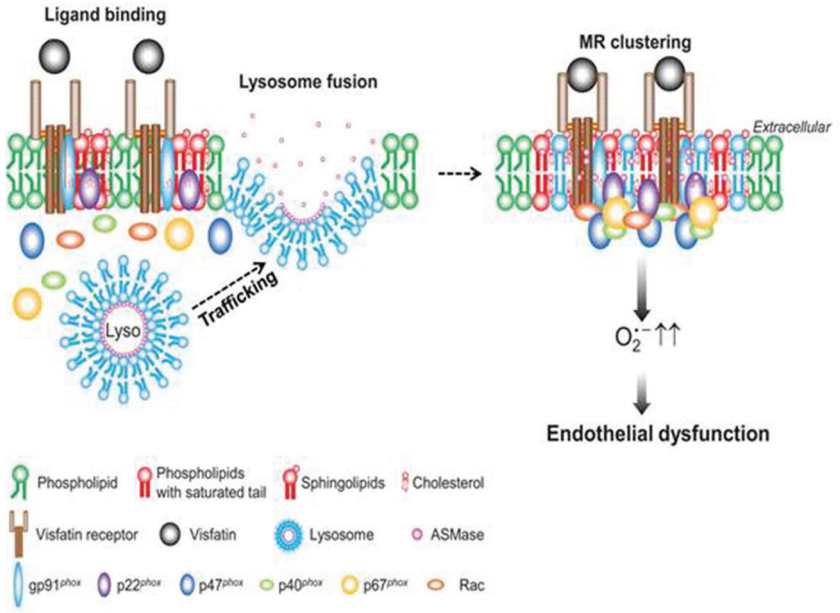

Figure 4. Diagram of visfatin-induced membrane raft clustering that results in the formation of membrane raft redox signalling platforms in coronary artery endothelial cells.

In this study, prevention of the blood pressure increase by myriocin is endothelium-dependent and occurs via restoration of eNOS phosphorylation at Ser1177 (66). Moreover, heterozygous deficiency of DES1 partially restored phosphorylation of eNOS, suggesting a major role of ceramide in NO production. A potential mechanism responsible for aggravation of endothelial dysfunction by ceramide is via ceramide-mediated activation of protein phosphatase 2A causing dephosphorylation of eNOS and AKT dissociation (66). These findings provide the mechanistic link between obesity-induced vascular dysfunction and ceramide.

In addition, we have recently demonstrated that ceramide associated MR redox signaling platforms and NADPH oxidase to be responsible for endothelial dysfunction induced by visfatin, an adipokine and a major injurious factor during obesity and associated diseases including diabetes, carotid and coronary atherosclerosis and chronic kidney disease (37). As a commonly used functional study, endothelium dependent vasodilation response in isolated perfused arteries was intensively tested. We found that visfatin induced MR clustering and thereby resulted in the formation of MR redox signalling platforms in coronary arterial endothelial cells (99). As shown in Figure 4, the transmembrane redox signaling mechanism associated with MR clustering may mediate the detrimental action of visfatin to induce endothelial dysfunction. When visfatin acts on endothelial cells, lysosomes are stimulated to traffic and fuse into the cell membrane, resulting in the activation of ASMase, production of ceramide, and formation of MR-NOX redox signaling platforms. This redox signaling platform contains a variety of signaling molecules which interact to form signalosomes, producing $O_2^{\bullet-}$ and inducing endothelial dysfunction. Thus MR–NOX redox signaling platform is a critical molecular mechanism mediating visfatin-induced endothelial dysfunction such as impairment of endothelium-dependent vasodilation in endothelial cells (99).

6. THERAPEUTIC INTERVENTIONS BY TARGETING SPHINGOLIPIDS

Given that sphingolipids are importantly involved in obesity and associated complications, targeting sphingolipids and ceramide mediated pathways may be a useful strategy in the development of new therapies to prevent or treat these diseases. Most tool compounds used to alter SPT, ASMase activity or the action of ceramide could be possible drug candidates.

6.1. Inhibition of serine palmitoyl transferase (SPT) activity

The mechanisms involved in ceramide-induced metabolic diseases has led to a number of potential therapeutic targets being proposed to tackle obesity and associated complications. However, it would be reasonable to speculate that preventing ceramide accumulation in response to elevated levels of circulating lipids or other stimuli that promote metabolic diseases may be beneficial in ameliorating deleterious effects. With this in mind, the most commonly studied molecular target involved in suppressing ceramide production is the

enzyme SPT, which catalyses the initial rate-limiting step in de novo ceramide synthesis (100, 101). Several potent inhibitors of SPT have been documented, although the most widely used is myriocin, a naturally occurring fungal metabolite isolated from Myriococcum albomyces (101). Several studies have reported that acute inhibition of SPT using myriocin ameliorates the loss of insulin-stimulated protein kinase B activation in cultures L6 or C2C12 myotubes caused by palmitate-driven ceramide synthesis. *In vivo* studies also demonstrated that myriocin attenuates protein kinase B inhibition in response to lipid infusion or HFD, as well as improving glucose tolerance and peripheral insulin sensitivity in obese ob/ob mice and Zucker Diabetic Fatty rats (29). As expected, these beneficial effects of myriocin are associated with reduced levels of circulating ceramide. The other inhibitors of de novo ceramide synthesis such as L-cycloserine (which also inhibits SPT) and fumonisin B1 (a DES1inhibitor) also decreased the plasma ceramide (29) and improved the insulin resistance. Furthermore, Yi *et al* reported that myriocin treatment decreased the renal cortex ceramide production and protected the glomeruli from hyperhomocysteinemia-induced injury (74). There is a possibility that myriocin treatment may act to simultaneously reduce levels of other sphingolipids derived from ceramide (e.g. glycosphingolipids), thereby contributing to its beneficial effects. However, such consequential responses of myriocin administration have not yet been reported.

As well as targeting SPT directly, there is also evidence to suggest that manipulating the activity of molecular targets or pathways that do not directly participate in the de novo synthesis of ceramide, may also result in the modulation of SPT activity and/or ceramide production. For example, adiponectin, an adipokine can act through its receptors (AdipoR1 and AdipoR2) to lower ceramide levels by stimulating the activity of acid ceramidase, an enzyme that catalyses the degradation of ceramide (102).

6.2. Inhibition of acid sphingomyelinase activity

Ceramide can also be produced by the action of SMase enzymes that are activated in response to stimuli such as visfatin or obesity. SMase generates ceramide through the hydrolysis of sphingomyelin (Figure 1). Interestingly, the abundance and/or activity of either neutral SMase or ASMase has been reported to be elevated in the adipose tissue of ob/ob and HFD-induced obese mice as well as in kidney in response to HFD feeding (24, 100). It is plausible that increased levels and/or activity of sphingomyelinase-stimulating stimuli or secreted serum SMase may also contribute to ceramide accumulation in peripheral tissues (103). Indeed, it has been shown that genetic loss of ASMase can prevent diet-induced obesity, hyperglycemia and insulin resistance in mice lacking the LDL receptor ($Ldlr^{-/-}$), which are prone to metabolic disease when placed on a HFD (103). Furthermore, we have demonstrated that treatment of mice with the ASMase inhibitor amitriptyline attenuates HFD-induced elevations in plasma ceramide and improves obesity, insulin sensitivity and glomerular injury (24). Taken together, these findings hold promise that SMases may be a therapeutic target for treatment of obesity and associated complications.

Using structure-property-activity models, chemists have characterized some organic weak bases as ASMase inhibitors that function by inducing a detachment of ASMase from inner lysosomal membranes and subsequent inactivation of the enzyme (104). Moreover, cationic amphiphilic substances can induce the detachment of ASMase proteins from inner lysosomal membranes, thereby inactivating them. These can be utilized as functional inhibitors of ASMase and are minimally toxic, which may be applied for disease states associated with increased activity of ASMase and ceramide-enriched platforms (105).

Recently, a potent and selective novel inhibitor of ASMase, L-alpha-phosphatidyl-D-myo-inositol-3, 5-bisphosphate (PtdIns3, 5P2), was reported. As a naturally occurring substance detected in mammalian, plant and yeast cells, it may also be used as starting point for the development of new potent ASMase inhibitors optimized for diverse applications (105). Based on many experimental results, inhibition of ASMase or gene silencing of ASMase genes can be an appropriate strategy for prevention or treatment of metabolic diseases. There is no compound known to us that is effective in inhibiting ASMase and available for clinical use. However, a group of German scientists led by Dr. Eric Gulbins introduced a large group of compounds with a broad range of new clinical indications, they named ‘‘FIASMA’’ (Functional Inhibitor of ASMase). FIASMAs differ markedly with respect to molecular structure and current clinical indications, and most of the available compounds of this group of ASMase inhibitors are licensed for medical use in humans, which are minimally toxic and may therefore be applied for disease states associated with increased activity of ASMase (105).

6.3. Targeting gangliosides

Another class of ceramide-derived sphingolipids that have been implicated as modulators of transmembrane signaling is the gangliosides. Gangliosides are sialic acid-containing glycosphingolipids consisting of ceramide moiety linked to an oligosaccharide chain (87). One of the gangliosides, in particular, ganglioside monosialo 3 (GM3) has been shown to increase in type 2 diabetics accompanied by severe visceral fat accumulation and modulates in insulin signaling. Data from several studies now suggest that GM3 may be involved in mediating insulin-desensitizing effects and, in particular, that of

pro-inflammatory cytokines such as TNFα (106). First, insulin resistance induced by TNFα in 3T3-L1 adipocytes has been associated with elevated the GM3 levels caused by increased GM3 synthase abundance and activity (106). Second, pharmacological inhibitors of glucosylceramide synthase, which deplete cellular GM3, can prevent the inhibitory effects of TNFα on insulin signaling in cultured 3T3-L1 adipocytes (106). Further, it was shown that expression of ganglioside GM3, which is the simplest ganglioside species synthesized by GM3 synthase (also called SAT-I/ST3Gal-5), is increased in metabolic diseases (106, 107). GM3 synthase gene expression and GM3 content are upregulated in visceral adipose tissue of obese animals and serum GM3 levels are approximately 2-fold higher in obese patients with type 2 diabetes and/or dyslipidemia (106, 107). Further supporting a role for GM3 in the development of insulin resistance *in vivo*, administration of glucosylceramide synthase inhibitors (N-(5-adamantane-1-yl-methoxypentyl)-deoxynojirimycin (AMP-DNM) and Genz-123346) has been shown to improve both glucose tolerance and insulin sensitivity in skeletal muscle and liver of ob/ob mice and Zucker diabetic rats, as well as in diet-induced obese mice, without any significant alteration in food intake or loss of body weight (61). Similar effects are observed in mice lacking GM3, which display protection against HFD-induced insulin resistance (58).

Clinical studies have shown increased circulating levels of GM3 in obese type 2 diabetic individuals displaying hyperglycemia and/or hyperlipidemia (107). Together, these observations open up the possibility that GM3 may serve as a diagnostic marker for metabolic-related disorders and that therapeutic interventions aimed at reducing GM3 levels may prove to be a useful strategy for combating metabolic diseases.

7. CONCLUSION

In conclusion, sphingolipids are supposed to play an important role in the regulation of many cellular functions. Experimental evidence from *in vitro* and *in vivo* studies suggest that pharmacological interference with sphingolipids may offer novel approaches for treatment and prevention of obesity and associated complications. While most experimental data are still far from being translated to clinical practice, modulation of the gaglioside GM3 in metabolic disease has already become a major clinical application of sphingolipid research in the last few years. However, it could be expected that future exploration of the role of sphingolipids in other metabolic diseases will become of similar clinical relevance.

8. ACKNOWLEDGEMENTS

Many studies cited in this review from our laboratory were supported by grants DK54927, HL091464, HL57244 and HL075316 (to P.L.) and DK104031 to (K.B.) from National Institutes of Health and VCU's CTSA (UL1TR000058 from the National Center for Advancing Translational Sciences) and the CCTR Endowment Fund of Virginia Commonwealth University (to K.B.).

9. REFERENCES

1. J. E. Shaw, R. A. Sicree and P. Z. Zimmet: Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract*, 87(1), 4-14 (2010) DOI: 10.1016/j.diabres.2009.10.007
2. G. Boden: Obesity and free fatty acids. *Endocrinol Metab Clin North Am*, 37(3), 635-46, viii-ix (2008)
3. N. T. Nguyen, C. P. Magno, K. T. Lane, M. W. Hinojosa and J. S. Lane: Association of hypertension, diabetes, dyslipidemia, and metabolic syndrome with obesity: findings from the National Health and Nutrition Examination Survey, 1999 to 2004. *J Am Coll Surg*, 207(6), 928-34 (2008) DOI: 10.1016/j.jamcollsurg.2008.08.022
4. M. N. Barber, S. Risis, C. Yang, P. J. Meikle, M. Staples, M. A. Febbraio and C. R. Bruce: Plasma lysophosphatidylcholine levels are reduced in obesity and type 2 diabetes. *PLoS One*, 7(7), e41456 (2012) DOI: 10.1371/journal.pone.0041456
5. F. Samad, K. D. Hester, G. Yang, Y. A. Hannun and J. Bielawski: Altered adipose and plasma sphingolipid metabolism in obesity: a potential mechanism for cardiovascular and metabolic risk. *Diabetes*, 55(9), 2579-87 (2006) DOI: 10.2337/db06-0330
6. M. J. Watt, A. C. Barnett, C. R. Bruce, S. Schenk, J. F. Horowitz and A. J. Hoy: Regulation of plasma ceramide levels with fatty acid oversupply: evidence that the liver detects and secretes de novo synthesised ceramide. *Diabetologia*, 55(10), 2741-6 (2012) DOI: 10.1007/s00125-012-2649-3
7. G. van Echten-Deckert and T. Herget: Sphingolipid metabolism in neural cells. *Biochim Biophys Acta*, 1758(12), 1978-94 (2006) DOI: 10.1016/j.bbamem.2006.06.009
8. T. Wennekes, R. J. van den Berg, R. G. Boot, G. A. van der Marel, H. S. Overkleeft and J. M.

Aerts: Glycosphingolipids--nature, function, and pharmacological modulation. *Angew Chem Int Ed Engl*, 48(47), 8848-69 (2009)
DOI: 10.1002/anie.200902620

9. Y. H. Zeidan and Y. A. Hannun: Translational aspects of sphingolipid metabolism. *Trends Mol Med*, 13(8), 327-36 (2007)
DOI: 10.1016/j.molmed.2007.06.002

10. S. Narayan and E. A. Thomas: Sphingolipid abnormalities in psychiatric disorders: a missing link in pathology? *Front Biosci (Landmark Ed)*, 16, 1797-810 (2011)
DOI: 10.2741/3822

11. Y. A. Hannun: Functions of ceramide in coordinating cellular responses to stress. *Science*, 274(5294), 1855-9 (1996)
DOI: 10.1126/science.274.5294.1855

12. C. Luberto, J. M. Kraveka and Y. A. Hannun: Ceramide regulation of apoptosis versus differentiation: a walk on a fine line. Lessons from neurobiology. *Neurochem Res*, 27(7-8), 609-17 (2002)
DOI: 10.1023/A:1020267831851

13. P. P. Ruvolo: Intracellular signal transduction pathways activated by ceramide and its metabolites. *Pharmacol Res*, 47(5), 383-92 (2003)
DOI: 10.1016/S1043-6618(03)00050-1

14. C. Garcia-Ruiz, A. Colell, M. Mari, A. Morales and J. C. Fernandez-Checa: Direct effect of ceramide on the mitochondrial electron transport chain leads to generation of reactive oxygen species. Role of mitochondrial glutathione. *J Biol Chem*, 272(17), 11369-77 (1997)
DOI: 10.1074/jbc.272.17.11369

15. E. H. Schuchman: The pathogenesis and treatment of acid sphingomyelinase-deficient Niemann-Pick disease. *Int J Clin Pharmacol Ther*, 47 Suppl 1, S48-57 (2009)
DOI: 10.5414/cpp47048

16. K. Ogawa-Goto and T. Abe: Gangliosides and glycosphingolipids of peripheral nervous system myelins--a minireview. *Neurochem Res*, 23(3), 305-10 (1998)
DOI: 10.1023/A:1022497114813

17. T. Kolter and K. Sandhoff: Sphingolipid metabolism diseases. *Biochim Biophys Acta*, 1758(12), 2057-79 (2006)
DOI: 10.1016/j.bbamem.2006.05.027

18. M. Eckhardt: The role and metabolism of sulfatide in the nervous system. *Mol Neurobiol*, 37(2-3), 93-103 (2008)
DOI: 10.1007/s12035-008-8022-3

19. E. Meacci, F. Cencetti, C. Donati, F. Nuti, L. Becciolini and P. Bruni: Sphingosine kinase activity is required for sphingosine-mediated phospholipase D activation in C2C12 myoblasts. *Biochem J*, 381(Pt 3), 655-63 (2004)
DOI: 10.1042/BJ20031636

20. M. N. Nikolova-Karakashian and M. B. Reid: Sphingolipid metabolism, oxidant signaling, and contractile function of skeletal muscle. *Antioxid Redox Signal*, 15(9), 2501-17 (2011)
DOI: 10.1089/ars.2011.3940

21. D. S. Wijesinghe, A. Massiello, P. Subramanian, Z. Szulc, A. Bielawska and C. E. Chalfant: Substrate specificity of human ceramide kinase. *J Lipid Res*, 46(12), 2706-16 (2005)
DOI: 10.1194/jlr.M500313-JLR200

22. E. Ikonen and S. Vainio: Lipid microdomains and insulin resistance: is there a connection? *Sci STKE*, 2005(268), pe3 (2005)
DOI: 10.1126/stke.2682005pe3

23. J. Inokuchi: Insulin resistance as a membrane microdomain disorder. *Biol Pharm Bull*, 29(8), 1532-7 (2006)
DOI: 10.1248/bpb.29.1532

24. K. M. Boini, C. Zhang, M. Xia, J. L. Poklis and P. L. Li: Role of sphingolipid mediator ceramide in obesity and renal injury in mice fed a high-fat diet. *J Pharmacol Exp Ther*, 334(3), 839-46 (2010)
DOI: 10.1124/jpet.110.168815

25. K. M. Boini, C. Zhang, M. Xia, W. Q. Han, C. Brimson, J. L. Poklis and P. L. Li: Visfatin-induced lipid raft redox signaling platforms and dysfunction in glomerular endothelial cells. *Biochim Biophys Acta*, 1801(12), 1294-304 (2010)
DOI: 10.1016/j.bbalip.2010.09.001

26. S. M. Walls, Jr., S. J. Attle, G. B. Brulte, M. L. Walls, K. D. Finley, D. A. Chatfield, D. R. Herr and G. L. Harris: Identification of sphingolipid metabolites that induce obesity via misregulation of appetite, caloric intake and fat storage in Drosophila. *PLoS Genet*, 9(12), e1003970 (2013)

DOI: 10.1371/journal.pgen.1003970

27. E. W. Kraegen, G. J. Cooney, J. M. Ye, A. L. Thompson and S. M. Furler: The role of lipids in the pathogenesis of muscle insulin resistance and beta cell failure in type II diabetes and obesity. *Exp Clin Endocrinol Diabetes*, 109 Suppl 2, S189-201 (2001)
DOI: 10.1055/s-2001-18581

28. R. H. Unger: Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. *Endocrinology*, 144(12), 5159-65 (2003)
DOI: 10.1210/en.2003-0870

29. W. L. Holland, J. T. Brozinick, L. P. Wang, E. D. Hawkins, K. M. Sargent, Y. Liu, K. Narra, K. L. Hoehn, T. A. Knotts, A. Siesky, D. H. Nelson, S. K. Karathanasis, G. K. Fontenot, M. J. Birnbaum and S. A. Summers: Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell Metab*, 5(3), 167-79 (2007)
DOI: 10.1016/j.cmet.2007.01.002

30. R. A. Memon, W. M. Holleran, A. H. Moser, T. Seki, Y. Uchida, J. Fuller, J. K. Shigenaga, C. Grunfeld and K. R. Feingold: Endotoxin and cytokines increase hepatic sphingolipid biosynthesis and produce lipoproteins enriched in ceramides and sphingomyelin. *Arterioscler Thromb Vasc Biol*, 18(8), 1257-65 (1998)
DOI: 10.1161/01.ATV.18.8.1257

31. W. Zheng, J. Kollmeyer, H. Symolon, A. Momin, E. Munter, E. Wang, S. Kelly, J. C. Allegood, Y. Liu, Q. Peng, H. Ramaraju, M. C. Sullards, M. Cabot and A. H. Merrill, Jr.: Ceramides and other bioactive sphingolipid backbones in health and disease: lipidomic analysis, metabolism and roles in membrane structure, dynamics, signaling and autophagy. *Biochim Biophys Acta*, 1758(12), 1864-84 (2006)
DOI: 10.1016/j.bbamem.2006.08.009

32. J. L. Halaas, K. S. Gajiwala, M. Maffei, S. L. Cohen, B. T. Chait, D. Rabinowitz, R. L. Lallone, S. K. Burley and J. M. Friedman: Weight-reducing effects of the plasma protein encoded by the obese gene. *Science*, 269(5223), 543-6 (1995)
DOI: 10.1126/science.7624777

33. Y. Minokoshi, M. S. Haque and T. Shimazu: Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. *Diabetes*, 48(2), 287-91 (1999)
DOI: 10.2337/diabetes.48.2.287

34. E. Bonzon-Kulichenko, D. Schwudke, N. Gallardo, E. Molto, T. Fernandez-Agullo, A. Shevchenko and A. Andres: Central leptin regulates total ceramide content and sterol regulatory element binding protein-1C proteolytic maturation in rat white adipose tissue. *Endocrinology*, 150(1), 169-78 (2009)
DOI: 10.1210/en.2008-0505

35. H. Ma, V. Gomez, L. Lu, X. Yang, X. Wu and S. Y. Xiao: Expression of adiponectin and its receptors in livers of morbidly obese patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol*, 24(2), 233-7 (2009)
DOI: 10.1111/j.1440-1746.2008.05548.x

36. C. K. Huang, R. Goel, P. C. Chang, C. H. Lo and A. Shabbir: Single-incision transumbilical (SITU) surgery after SITU laparoscopic Roux-en-Y gastric bypass. *J Laparoendosc Adv Surg Tech A*, 22(8), 764-7 (2012)
DOI: 10.1089/lap.2011.0434

37. M. Xia, K. M. Boini, J. M. Abais, M. Xu, Y. Zhang and P. L. Li: Endothelial NLRP3 inflammasome activation and enhanced neointima formation in mice by adipokine visfatin. *Am J Pathol*, 184(5), 1617-28 (2014)
DOI: 10.1016/j.ajpath.2014.01.032

38. S. A. Summers: Ceramides in insulin resistance and lipotoxicity. *Prog Lipid Res*, 45(1), 42-72 (2006)
DOI: 10.1016/j.plipres.2005.11.002

39. J. M. Abais, M. Xia, Y. Zhang, K. M. Boini and P. L. Li: Redox regulation of NLRP3 inflammasomes: ROS as trigger or effector? *Antioxid Redox Signal*, 22(13), 1111-29 (2015)
DOI: 10.1089/ars.2014.5994

40. B. Vandanmagsar, Y. H. Youm, A. Ravussin, J. E. Galgani, K. Stadler, R. L. Mynatt, E. Ravussin, J. M. Stephens and V. D. Dixit: The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat Med*, 17(2), 179-88 (2011)
DOI: 10.1038/nm.2279

41. K. M. Boini, M. Xia, J. M. Abais, G. Li, A. L. Pitzer, T. W. Gehr, Y. Zhang and P. L. Li: Activation of inflammasomes in podocyte

injury of mice on the high fat diet: Effects of ASC gene deletion and silencing. *Biochim Biophys Acta*, 1843(5), 836-45 (2014)
DOI: 10.1016/j.bbamcr.2014.01.033

42. Y. Lee, H. Hirose, M. Ohneda, J. H. Johnson, J. D. McGarry and R. H. Unger: Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. *Proc Natl Acad Sci U S A*, 91(23), 10878-82 (1994)
DOI: 10.1073/pnas.91.23.10878

43. H. Malhi and G. J. Gores: Molecular mechanisms of lipotoxicity in nonalcoholic fatty liver disease. *Semin Liver Dis*, 28(4), 360-9 (2008)
DOI: 10.1055/s-0028-1091980

44. N. A. Bourbon, L. Sandirasegarane and M. Kester: Ceramide-induced inhibition of Akt is mediated through protein kinase Czeta: implications for growth arrest. *J Biol Chem*, 277(5), 3286-92 (2002)
DOI: 10.1074/jbc.M110541200

45. C. Shah, G. Yang, I. Lee, J. Bielawski, Y. A. Hannun and F. Samad: Protection from high fat diet-induced increase in ceramide in mice lacking plasminogen activator inhibitor 1. *J Biol Chem*, 283(20), 13538-48 (2008)
DOI: 10.1074/jbc.M709950200

46. B. Liu, L. M. Obeid and Y. A. Hannun: Sphingomyelinases in cell regulation. *Semin Cell Dev Biol*, 8(3), 311-322 (1997)
DOI: 10.1006/scdb.1997.0153

47. Z. Li, M. J. Basterr, T. K. Hailemariam, M. R. Hojjati, S. Lu, J. Liu, R. Liu, H. Zhou and X. C. Jiang: The effect of dietary sphingolipids on plasma sphingomyelin metabolism and atherosclerosis. *Biochim Biophys Acta*, 1735(2), 130-4 (2005)
DOI: 10.1016/j.bbalip.2005.05.004

48. T. S. Park, 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: Inhibition of sphingomyelin synthesis reduces atherogenesis in apolipoprotein E-knockout mice. *Circulation*, 110(22), 3465-71 (2004)
DOI: 10.1161/01.CIR.0000148370.60535.22

49. J. M. Holopainen, J. Y. Lehtonen and P. K. Kinnunen: Lipid microdomains in dimyristoylphosphatidylcholine-ceramide liposomes. *Chem Phys Lipids*, 88(1), 1-13 (1997)
DOI: 10.1016/S0009-3084(97)00040-6

50. S. L. Schissel, J. Tweedie-Hardman, J. H. Rapp, G. Graham, K. J. Williams and I. Tabas: 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*, 98(6), 1455-64 (1996)
DOI: 10.1172/JCI118934

51. S. Y. Morita, K. Okuhira, N. Tsuchimoto, A. Vertut-Doi, H. Saito, M. Nakano and T. Handa: Effects of sphingomyelin on apolipoprotein E- and lipoprotein lipase-mediated cell uptake of lipid particles. *Biochim Biophys Acta*, 1631(2), 169-76 (2003)
DOI: 10.1016/S1388-1981(02)00365-7

52. Y. Yatomi, T. Ohmori, G. Rile, F. Kazama, H. Okamoto, T. Sano, K. Satoh, S. Kume, G. Tigyi, Y. Igarashi and Y. Ozaki: Sphingosine 1-phosphate as a major bioactive lysophospholipid that is released from platelets and interacts with endothelial cells. *Blood*, 96(10), 3431-8 (2000)

53. Q. Yang, T. E. Graham, N. Mody, F. Preitner, O. D. Peroni, J. M. Zabolotny, K. Kotani, L. Quadro and B. B. Kahn: Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature*, 436(7049), 356-62 (2005)
DOI: 10.1038/nature03711

54. J. Boon, A. J. Hoy, R. Stark, R. D. Brown, R. C. Meex, D. C. Henstridge, S. Schenk, P. J. Meikle, J. F. Horowitz, B. A. Kingwell, C. R. Bruce and M. J. Watt: Ceramides contained in LDL are elevated in type 2 diabetes and promote inflammation and skeletal muscle insulin resistance. *Diabetes*, 62(2), 401-10 (2013)
DOI: 10.2337/db12-0686

55. X. Lopez, A. B. Goldfine, W. L. Holland, R. Gordillo and P. E. Scherer: Plasma ceramides are elevated in female children and adolescents with type 2 diabetes. *J Pediatr Endocrinol Metab*, 26(9-10), 995-8 (2013)
DOI: 10.1515/jpem-2012-0407

56. H. Huang, T. Kasumov, P. Gatmaitan, H. M. Heneghan, S. R. Kashyap, P. R. Schauer,

S. A. Brethauer and J. P. Kirwan: Gastric bypass surgery reduces plasma ceramide subspecies and improves insulin sensitivity in severely obese patients. *Obesity (Silver Spring)*, 19(11), 2235-40 (2011)
DOI: 10.1038/oby.2011.107

57. W. Truong, J. A. Emamaullee, S. Merani, C. C. Anderson and A. M. James Shapiro: Human islet function is not impaired by the sphingosine-1-phosphate receptor modulator FTY720. *Am J Transplant*, 7(8), 2031-8 (2007)
DOI: 10.1111/j.1600-6143.2007.01880.x

58. T. Yamashita, A. Hashiramoto, M. Haluzik, H. Mizukami, S. Beck, A. Norton, M. Kono, S. Tsuji, J. L. Daniotti, N. Werth, R. Sandhoff, K. Sandhoff and R. L. Proia: Enhanced insulin sensitivity in mice lacking ganglioside GM3. *Proc Natl Acad Sci U S A*, 100(6), 3445-9 (2003)
DOI: 10.1073/pnas.0635898100

59. J. M. Aerts, R. Ottenhoff, A. S. Powlson, A. Grefhorst, M. van Eijk, P. F. Dubbelhuis, J. Aten, F. Kuipers, M. J. Serlie, T. Wennekes, J. K. Sethi, S. O'Rahilly and H. S. Overkleeft: Pharmacological inhibition of glucosylceramide synthase enhances insulin sensitivity. *Diabetes*, 56(5), 1341-9 (2007)
DOI: 10.2337/db06-1619

60. L. Lee, A. Abe and J. A. Shayman: Improved inhibitors of glucosylceramide synthase. *J Biol Chem*, 274(21), 14662-9 (1999)
DOI: 10.1074/jbc.274.21.14662

61. H. Zhao, M. Przybylska, I. H. Wu, J. Zhang, C. Siegel, S. Komarnitsky, N. S. Yew and S. H. Cheng: Inhibiting glycosphingolipid synthesis improves glycemic control and insulin sensitivity in animal models of type 2 diabetes. *Diabetes*, 56(5), 1210-8 (2007)
DOI: 10.2337/db06-0719

62. J. M. Haus, S. R. Kashyap, T. Kasumov, R. Zhang, K. R. Kelly, R. A. Defronzo and J. P. Kirwan: Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes*, 58(2), 337-43 (2009)
DOI: 10.2337/db08-1228

63. E. Hajduch, A. Balendran, I. H. Batty, G. J. Litherland, A. S. Blair, C. P. Downes and H. S. Hundal: Ceramide impairs the insulin-dependent membrane recruitment of protein kinase B leading to a loss in downstream signalling in L6 skeletal muscle cells. *Diabetologia*, 44(2), 173-83 (2001)
DOI: 10.1007/s001250051596

64. S. A. Summers, L. A. Garza, H. Zhou and M. J. Birnbaum: Regulation of insulin-stimulated glucose transporter GLUT4 translocation and Akt kinase activity by ceramide. *Mol Cell Biol*, 18(9), 5457-64 (1998)
DOI: 10.1128/MCB.18.9.5457

65. D. J. Powell, S. Turban, A. Gray, E. Hajduch and H. S. Hundal: Intracellular ceramide synthesis and protein kinase Czeta activation play an essential role in palmitate-induced insulin resistance in rat L6 skeletal muscle cells. *Biochem J*, 382(Pt 2), 619-29 (2004)
DOI: 10.1042/BJ20040139

66. Q. J. Zhang, W. L. Holland, L. Wilson, J. M. Tanner, D. Kearns, J. M. Cahoon, D. Pettey, J. Losee, B. Duncan, D. Gale, C. A. Kowalski, N. Deeter, A. Nichols, M. Deesing, C. Arrant, T. Ruan, C. Boehme, D. R. McCamey, J. Rou, K. Ambal, K. K. Narra, S. A. Summers, E. D. Abel and J. D. Symons: Ceramide mediates vascular dysfunction in diet-induced obesity by PP2A-mediated dephosphorylation of the eNOS-Akt complex. *Diabetes*, 61(7), 1848-59 (2012)
DOI: 10.2337/db11-1399

67. L. J. Spijkers, R. F. van den Akker, B. J. Janssen, J. J. Debets, J. G. De Mey, E. S. Stroes, B. J. van den Born, D. S. Wijesinghe, C. E. Chalfant, L. MacAleese, G. B. Eijkel, R. M. Heeren, A. E. Alewijnse and S. L. Peters: Hypertension is associated with marked alterations in sphingolipid biology: a potential role for ceramide. *PLoS One*, 6(7), e21817 (2011)
DOI: 10.1371/journal.pone.0021817

68. C. Berry, R. Touyz, A. F. Dominiczak, R. C. Webb and D. G. Johns: Angiotensin receptors: signaling, vascular pathophysiology, and interactions with ceramide. *Am J Physiol Heart Circ Physiol*, 281(6), H2337-65 (2001)

69. R. M. Fryer, A. Muthukumarana, P. C. Harrison, S. Nodop Mazurek, R. R. Chen, K. E. Harrington, R. M. Dinallo, J. C. Horan, L. Patnaude, L. K. Modis and G. A. Reinhart: The clinically-tested S1P receptor agonists, FTY720 and BAF312, demonstrate subtype-specific bradycardia (S1P(1)) and hypertension (S1P(3)) in rat. *PLoS One*, 7(12), e52985 (2012)

DOI: 10.1371/journal.pone.0052985

70. M. Czarny and J. E. Schnitzer: Neutral sphingomyelinase inhibitor scyphostatin prevents and ceramide mimics mechanotransduction in vascular endothelium. *Am J Physiol Heart Circ Physiol*, 287(3), H1344-52 (2004)
DOI: 10.1152/ajpheart.00222.2004

71. M. Fenger, A. Linneberg, T. Jorgensen, S. Madsbad, K. Sobye, J. Eugen-Olsen and J. Jeppesen: Genetics of the ceramide/sphingosine-1-phosphate rheostat in blood pressure regulation and hypertension. *BMC Genet*, 12, 44 (2011)
DOI: 10.1186/1471-2156-12-44

72. G. P. Kaushal, A. B. Singh and S. V. Shah: Identification of gene family of caspases in rat kidney and altered expression in ischemia-reperfusion injury. *Am J Physiol*, 274(3 Pt 2), F587-95 (1998)

73. N. Ueda, G. P. Kaushal and S. V. Shah: Apoptotic mechanisms in acute renal failure. *Am J Med*, 108(5), 403-15 (2000)
DOI: 10.1016/S0002-9343(00)00311-9

74. F. Yi, A. Y. Zhang, J. L. Janscha, P. L. Li and A. P. Zou: Homocysteine activates NADH/NADPH oxidase through ceramide-stimulated Rac GTPase activity in rat mesangial cells. *Kidney Int*, 66(5), 1977-87 (2004)
DOI: 10.1111/j.1523-1755.2004.00968.x

75. T. Yin, G. Sandhu, C. D. Wolfgang, A. Burrier, R. L. Webb, D. F. Rigel, T. Hai and J. Whelan: Tissue-specific pattern of stress kinase activation in ischemic/reperfused heart and kidney. *J Biol Chem*, 272(32), 19943-50 (1997)
DOI: 10.1074/jbc.272.32.19943

76. W. Liu, T. Lan, X. Xie, K. Huang, J. Peng, J. Huang, X. Shen, P. Liu and H. Huang: S1P2 receptor mediates sphingosine-1-phosphate-induced fibronectin expression via MAPK signaling pathway in mesangial cells under high glucose condition. *Exp Cell Res*, 318(8), 936-43 (2012)
DOI: 10.1016/j.yexcr.2012.02.020

77. R. L. Klein, S. M. Hammad, N. L. Baker, K. J. Hunt, M. M. Al Gadban, P. A. Cleary, G. Virella, M. F. Lopes-Virella and D. E. R. Group: Decreased plasma levels of select very long chain ceramide species are associated with the development of nephropathy in type 1 diabetes. *Metabolism*, 63(10), 1287-95 (2014)
DOI: 10.1016/j.metabol.2014.07.001

78. R. Ross: The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*, 362(6423), 801-9 (1993)
DOI: 10.1038/362801a0

79. J. L. Witztum and D. Steinberg: Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest*, 88(6), 1785-92 (1991)
DOI: 10.1172/JCI115499

80. S. Yla-Herttuala, W. Palinski, M. E. Rosenfeld, S. Parthasarathy, T. E. Carew, S. Butler, J. L. Witztum and D. Steinberg: Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J Clin Invest*, 84(4), 1086-95 (1989)
DOI: 10.1172/JCI114271

81. H. A. Newman, C. E. Mc and D. B. Zilversmit: The synthesis of C14-lipids in rabbit atheromatous lesions. *J Biol Chem*, 236, 1264-8 (1961)

82. O. W. Portman and D. R. Illingworth: Arterial metabolism in primates. *Primates Med*, 9, 145-223 (1976)

83. E. B. Smith: Intimal and medial lipids in human aortas. *Lancet*, 1(7128), 799-803 (1960)
DOI: 10.1016/S0140-6736(60)90680-2

84. J. R. Guyton and K. F. Klemp: Development of the lipid-rich core in human atherosclerosis. *Arterioscler Thromb Vasc Biol*, 16(1), 4-11 (1996)
DOI: 10.1161/01.ATV.16.1.4

85. S. Eisenberg, Y. Stein and O. Stein: 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*, 48(12), 2320-9 (1969)
DOI: 10.1172/JCI106198

86. D. B. Zilversmit, C. E. Mc, P. H. Jordan, W. S. Henly and R. F. Ackerman: The synthesis of phospholipids in human atheromatous lesions. *Circulation*, 23, 370-5 (1961)
DOI: 10.1161/01.CIR.23.3.370

87. T. Jeong, S. L. Schissel, I. Tabas, H. J. Pownall, A. R. Tall and X. Jiang: 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(4), 905-12 (1998)
DOI: 10.1172/JCI870

88. A. S. Plump, J. D. Smith, T. Hayek, K. Aalto-Setala, A. Walsh, J. G. Verstuyft, E. M. Rubin and J. L. Breslow: Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell*, 71(2), 343-53 (1992)
DOI: 10.1016/0092-8674(92)90362-G

89. S. H. Zhang, R. L. Reddick, J. A. Piedrahita and N. Maeda: Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science*, 258(5081), 468-71 (1992)
DOI: 10.1126/science.1411543

90. X. C. Jiang, F. Paultre, T. A. Pearson, R. G. Reed, C. K. Francis, M. Lin, L. Berglund and A. R. Tall: Plasma sphingomyelin level as a risk factor for coronary artery disease. *Arterioscler Thromb Vasc Biol*, 20(12), 2614-8 (2000)
DOI: 10.1161/01.ATV.20.12.2614

91. T. S. Park, W. Rosebury, E. K. Kindt, M. C. Kowala and R. L. Panek: Serine palmitoyltransferase inhibitor myriocin induces the regression of atherosclerotic plaques in hyperlipidemic ApoE-deficient mice. *Pharmacol Res*, 58(1), 45-51 (2008)
DOI: 10.1016/j.phrs.2008.06.005

92. E. N. Glaros, W. S. Kim and B. Garner: Myriocin-mediated up-regulation of hepatocyte apoA-I synthesis is associated with ERK inhibition. *Clin Sci (Lond)*, 118(12), 727-36 (2010)
DOI: 10.1042/CS20090452

93. R. Klingenberg, J. R. Nofer, M. Rudling, F. Bea, E. Blessing, M. Preusch, H. J. Grone, H. A. Katus, G. K. Hansson and T. J. Dengler: Sphingosine-1-phosphate analogue FTY720 causes lymphocyte redistribution and hypercholesterolemia in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol*, 27(11), 2392-9 (2007)
DOI: 10.1161/ATVBAHA.107.149476

94. H. O. Steinberg, G. Paradisi, G. Hook, K. Crowder, J. Cronin and A. D. Baron: Free fatty acid elevation impairs insulin-mediated vasodilation and nitric oxide production. *Diabetes*, 49(7), 1231-8 (2000)
DOI: 10.2337/diabetes.49.7.1231

95. J. D. Symons, S. L. McMillin, C. Riehle, J. Tanner, M. Palionyte, E. Hillas, D. Jones, R. C. Cooksey, M. J. Birnbaum, D. A. McClain, Q. J. Zhang, D. Gale, L. J. Wilson and E. D. Abel: Contribution of insulin and Akt1 signaling to endothelial nitric oxide synthase in the regulation of endothelial function and blood pressure. *Circ Res*, 104(9), 1085-94 (2009)
DOI: 10.1161/CIRCRESAHA.108.189316

96. W. K. Alderton, C. E. Cooper and R. G. Knowles: Nitric oxide synthases: structure, function and inhibition. *Biochem J*, 357(Pt 3), 593-615 (2001)
DOI: 10.1042/0264-6021:3570593
DOI: 10.1042/bj3570593

97. J. A. Chavez and S. A. Summers: Lipid oversupply, selective insulin resistance, and lipotoxicity: molecular mechanisms. *Biochim Biophys Acta*, 1801(3), 252-65 (2010)
DOI: 10.1016/j.bbalip.2009.09.015

98. Y. Wu, P. Song, J. Xu, M. Zhang and M. H. Zou: Activation of protein phosphatase 2A by palmitate inhibits AMP-activated protein kinase. *J Biol Chem*, 282(13), 9777-88 (2007)
DOI: 10.1074/jbc.M608310200

99. M. Xia, C. Zhang, K. M. Boini, A. M. Thacker and P. L. Li: Membrane raft-lysosome redox signalling platforms in coronary endothelial dysfunction induced by adipokine visfatin. *Cardiovasc Res*, 89(2), 401-9 (2011)
DOI: 10.1093/cvr/cvq286

100. C. Lipina and H. S. Hundal: Sphingolipids: agents provocateurs in the pathogenesis of insulin resistance. *Diabetologia*, 54(7), 1596-607 (2011)
DOI: 10.1007/s00125-011-2127-3

101. K. Hanada: Serine palmitoyltransferase, a key enzyme of sphingolipid metabolism. *Biochim Biophys Acta*, 1632(1-3), 16-30 (2003)
DOI: 10.1016/S1388-1981(03)00059-3

102. W. L. Holland, R. A. Miller, Z. V. Wang, K. Sun, B. M. Barth, H. H. Bui, K. E. Davis, B. T. Bikman, N. Halberg, J. M. Rutkowski, M. R. Wade, V. M. Tenorio, M. S. Kuo, J. T. Brozinick, B. B. Zhang, M. J. Birnbaum, S. A. Summers and P. E. Scherer: Receptor-mediated activation

of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nat Med*, 17(1), 55-63 (2011)
DOI: 10.1038/nm.2277

103. G. M. Deevska, K. A. Rozenova, N. V. Giltiay, M. A. Chambers, J. White, B. B. Boyanovsky, J. Wei, A. Daugherty, E. J. Smart, M. B. Reid, A. H. Merrill, Jr. and M. Nikolova-Karakashian: Acid Sphingomyelinase Deficiency Prevents Diet-induced Hepatic Triacylglycerol Accumulation and Hyperglycemia in Mice. *J Biol Chem*, 284(13), 8359-68 (2009)
DOI: 10.1074/jbc.M807800200

104. J. Kornhuber, P. Tripal, M. Reichel, L. Terfloth, S. Bleich, J. Wiltfang and E. Gulbins: Identification of new functional inhibitors of acid sphingomyelinase using a structure-property-activity relation model. *J Med Chem*, 51(2), 219-37 (2008)
DOI: 10.1021/jm070524a

105. J. Kornhuber, P. Tripal, M. Reichel, C. Muhle, C. Rhein, M. Muehlbacher, T. W. Groemer and E. Gulbins: Functional Inhibitors of Acid Sphingomyelinase (FIASMAs): a novel pharmacological group of drugs with broad clinical applications. *Cell Physiol Biochem*, 26(1), 9-20 (2010)
DOI: 10.1159/000315101

106. S. Tagami, J. Inokuchi Ji, K. Kabayama, H. Yoshimura, F. Kitamura, S. Uemura, C. Ogawa, A. Ishii, M. Saito, Y. Ohtsuka, S. Sakaue and Y. Igarashi: Ganglioside GM3 participates in the pathological conditions of insulin resistance. *J Biol Chem*, 277(5), 3085-92 (2002)
DOI: 10.1074/jbc.M103705200

107. T. Sato, Y. Nihei, M. Nagafuku, S. Tagami, R. Chin, M. Kawamura, S. Miyazaki, M. Suzuki, S. Sugahara, Y. Takahashi, A. Saito, Y. Igarashi and J. Inokuchi: Circulating levels of ganglioside GM3 in metabolic syndrome: A pilot study. *Obes Res Clin Pract*, 2(4), I-II (2008)
DOI: 10.1016/j.orcp.2008.06.001

Key Words: Sphingolipids, Obesity, Ceramide, Metabolic Diseases, Review

Send correspondence to: Pin-Lan Li, Department of Pharmacology and Toxicology, Medical College of Virginia Campus, Virginia Commonwealth University, 410 N, 12th Street, Richmond, VA, 23298, Tel: 804-828 4793, Fax: 804-828-4794, E-mail: pli@vcu.edu