Compounds of the sphingomyelin-ceramide-glycosphingolipid pathways as secondary messenger molecules: new targets for novel therapies for fatty liver disease and insulin resistance

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Ilan Y. Compounds of the sphingomyelin-ceramide-glycosphingolipid pathways as secondary messenger molecules: new targets for novel therapies for fatty liver disease and insulin resistance. Am J Physiol Gastrointest Liver Physiol 310: G1102–G1117, 2016. First published May 12, 2016; doi:10.1152/ajpgi.00095.2016.—The compounds of sphingomyelin-ceramide-glycosphingolipid pathways have been studied as potential secondary messenger molecules in various systems, along with liver function and insulin resistance. Secondary messenger molecules act directly or indirectly to affect cell organelles and intercellular interactions. Their potential role in the pathogenesis of steatohepatitis and diabetes has been suggested. Data samples collected from patients with Gaucher’s disease, who had high levels of glucocerebroside, support a role for compounds from these pathways as a messenger molecule in the pathogenesis of fatty liver disease and diabetes. The present review summarizes some of the recent data on the role of glycosphingolipid molecules as messenger molecules in various physiological and pathological conditions, more specifically including insulin resistance and fatty liver disease.

NASH; NAFLD; glycosphingolipids; insulin resistance; β-glucosylceramide; Gaucher disease

SECONDARY MESSENGER MOLECULES act directly or indirectly to affect cellular and subcellular organelles. These molecules also determine intercellular cross talk. Potential secondary messenger molecules are explored in immunology as therapeutic targets. Compounds of the sphingomyelin (SM)-ceramide (Cer)-glycosphingolipid (GSL) pathways are studied as a potential secondary messenger molecules in various systems, and their potential role in pathological conditions, including steatohepatitis and the states of insulin resistance, is being investigated. Data collected from preclinical models and clinical data also collected from patients with fatty liver disease, diabetes, and Gaucher’s disease (GD), who have a high levels of glucocerebroside (GC), support a role for GSLs in the pathogenesis of these conditions. The present review summarizes some of the latest data on the role of these messenger molecules in various physiological and pathological conditions, mainly those that are related to insulin resistance and fatty liver disease, indicating they may be novel therapeutic targets.

Sphingolipid Steady State in Health and Disease

Sphingolipids (SLs) are lipids in which fatty acids are linked through amide bonds to a long-chain base. These molecules have effect in signal transduction and in the pathogenesis of several diseases (1, 2, 76, 80, 92, 96). Cer is the simplest molecule in this family, and it serves as a precursor for the synthesis of the three main types of complex SLs (SMs, GSLs, and gangliosides), respectively. Bioactive SLs, together with Cer, sphingosine (SPH), and sphingosine-1-phosphate (S1P), are secondary messenger molecules that regulate a diverse array of intra- and intercellular processes (3, 56, 60). They are important structural blocks because of their bioactive secondary messenger molecules in which they function in a different pattern of cellular processes.

GSLs are membrane components that act as a secondary messengers and/or modulators of signal transduction by affecting numerous cellular events, such as cell homeostasis, cell adhesion, cell growth, cell motility, apoptosis, cell cycle, cell proliferation, differentiation, angiogenesis, stress, and inflammatory responses (4, 5, 44, 46, 52, 65, 69, 130, 151). GSLs functions are linked to various aspects of cancer, i.e., tumor growth, neuroangiogenesis, and response to chemotherapy (5, 12, 44, 46, 54). Therapeutic strategies are emerging based on the design of small molecules, such as enzyme inhibitors or GSLs that can disrupt SL metabolic pathways to restore or alter their levels to the physiological condition, and SL analogs, to change the SL balance (11, 12, 42, 44, 153, 157). S1P acts intracellularly and extracellularly to promote calcium mobilization, intracellular signaling events, cytoskeleton rearrangements, and mitogenesis (11, 57). Sphingosine kinase is important for 1) revascularization responses, 2) regulating the maturation of vascular endothelial progenitors, and 3) controlling cellular recruitment (15, 85, 89). The cellular balance of the level of Cer, SPH, and S1P, termed the “sphingolipid rheostat,” dictates cell fate, whereby Cer and SPH enhance apoptosis while the S1P promotes cell survival and proliferation (16, 125,
However, the actual sphingolipid rheostat may be broad, comprising numerous additional molecules in the SL metabolic map as well as those in related metabolic pathways.

Figure 1A shows a schematic representation of the SM-Cer-GSL pathways. Each of the components of these pathways serves as a secondary messenger molecule under physiological or pathological states. Inherent to these pathways is a physiological effort to maintain a balance between all the elements. As shown in Fig. 1, B and C, all the compounds in these systems are interconnected, and it is expected that any changes in one of the elements will affect the others. Therefore, the blockade or induction of an enzyme can result in increase and/or decrease in upstream and downstream molecules to stimulate a new steady state. The new steady state is expected to have an effect on various systems, leading to a “new physiological state” or, in certain conditions, to a pathological disorder. In some pathologies, alterations in one or more of these secondary messenger molecules occur either as part of the primary hit or as a result of a secondary hit underlying a specific disease, making them novel targets for therapies.

**SL Pathways in Diabetes, NAFLD, and NASH**

**Role of GSLs in NASH pathogenesis.** Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are the most common causes of liver disorders. Both metabolic changes and inflammatory pathways are altered in these patients (10, 17, 18, 109, 113, 116, 120). Patients with NASH committed to inflammation early in the disease and continue to have progressive inflammation over time, leading to fibrogenesis and liver cirrhosis. A shift in one or more of the components of the SM-Cer-GSL pathways is associated with the pathogenesis, either as an initiator or evolving from them. Alternatively, alterations in one or more of the secondary molecules of these pathways may be involved in the process of the induction of liver damage and diabetes, as shown schematically in Fig. 2. A decrease or increase in one or more of the compounds in the SL pathways is likely to affect other molecules, which results to a new final steady state. The new SL balance then determines the target organ pathologies in the liver, pancreas, adipose tissue, and muscle, hence making the constituents of the SL pathway potential therapeutic targets. A pharmacological decrease or increase in one or more of the secondary messenger molecules of these pathways may be associated with restoring a normal balance of these molecules. Figure 1, B and C, illustrates various potential targets for pharmacological interventions in these pathways for reinduction of a steady state.

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**Fig. 1.** A: schematic presentation of the sphingomyelin-ceramide-glycosphingolipid pathways. B and C: potential sphingolipid-associated targets for pharmacological intervention for the treatment of fatty liver disease and diabetes.
The molecular mechanisms governing the transition from steatosis to steatohepatitis are not fully understood. Fat quantity determines disease advancement and studies have suggested that this type of fat is important for the development of steatohepatitis (19, 106, 110). The adipose tissue actively synthesizes and secretes cytokines; therefore, obesity-induced inflammation plays a role in the pathogenesis of disease states, including cardiovascular disease, NAFLD, and Type 2 diabetes. SLs are also relevant in the pathogenesis of these diseases. Obesity-related inflammatory pathways modulate SL metabolism (20, 23, 24). Metabolomics profiling, liquid chromatography mass spectrometry, and gene expression analyses showed increased hepatic Cer and a higher levels of SLs in NASH (21, 22, 28, 30, 36, 38). The metabolism of SL is dysregulated in obesity, in which increased adipose tissue mass affects whole body insulin resistance and cardiovascular disease risk via adipose-tissue derived inflammatory cytokines that induce chronic inflammation, as well as increasing cardiovascular risk while the antagonizing insulin signaling and mitochondrial function, thereby impairing glucose homeostasis (23, 149, 153). These data suggest that SLs are effective targets for the treatment of diseases associated with chronic inflammation (23, 20, 24).

Role for ceramides in NASH. Cers can affect cellular proliferation, differentiation, cell death, insulin resistance, oxidative stress, inflammation, and apoptosis, all of which are linked to NAFLD (24, 132, 136). De novo Cer synthesis plays a role in liver metabolism and has been investigated in the context of hepatic insulin resistance, NASH, obesity, dyslipidemia, hypertension, and cardiovascular diseases (25, 72, 76). Saturated fatty acids (SFAs) activate TLR-4 signaling, leading to the activation of IkB kinase, upregulation of de novo Cer biosynthesis, and Cer-induced activation of protein phosphatase 2A, which inhibits insulin signaling at the level of protein kinase B (Akt) phosphorylation (26–28, 32, 34, 53, 57, 73, 77). Palmitic acid is a precursor of Cer synthesized in the endoplasmic reticulum (ER), and it has been proposed that de novo Cer biosynthesis plays a role in SFA palmitic acid mediated lipotoxicity, and it is possible that a link exists between hepatocyte toxicity during ER stress, palmitic acid, and de novo Cer generation (28, 53, 57).

**GSLs and adiponectin.** Adiponectin secreted from adipocytes promotes insulin sensitivity and decreases inflammation (8, 9, 29). It stimulates a ceramidase activity that is linked to its two receptors, AdipoR1 and AdipoR2. Adiponectin increases Cer catabolism, resulting to increased formation of an anti-apoptotic metabolite, sphingosine-1-phosphate (S1P). This catabolic process is independent of AMP-dependent kinase (AMPK) (30, 74, 78). Overproduction of adiponectin decreases caspase-8-mediated death, whereas genetic ablation of adiponectin enhances apoptosis in vivo through a sphingolipid-mediated pathway.

**Role of GSLs in insulin signaling.** A regulatory interaction of GSLs with insulin signaling has been demonstrated (4, 5, 31). The insulin receptor is localized at the cell surface in a GSL-containing lipid microdomain (32, 93, 97). Cer, GC, ganglioside GM3, and other SLs are related to insulin resistance, pancreatic β-cell failure, and vascular dysfunction (33, 75, 79). Both Cer and GC are minor components of the lipid milieu in most tissues and they are potential pathogenic lipids in several conditions, including states of excess adiposity (24, 25, 33, 34, 75, 79). GSLs are structural membrane components located mainly in the plasma membrane, with their sugar moieties exposed at the cell surface (4, 5, 31). Cer, GC, and GM3 modify several steps of the insulin signaling pathway, such as phosphorylation with Akt or the insulin receptor (35, 135, 139). Cer-acyl chain length is important in insulin signaling, and TNF-α has been suggested as a secondary messenger to some of these functions.

**GSLs as biomarkers in obesity, diabetes, and NASH.** Serum SLs are obvious disease biomarkers in obesity and diabetes (36, 119, 123). Altered plasma and adipose tissue SL metabolism during obesity contribute to the prothrombotic and pro-inflammatory phenotype of obese adipose tissue; hence they are potential mechanisms for cardiovascular and metabolic disease risk (37, 150, 154). Adipose tissue SL metabolism is altered in genetically obese (ob/ob) mice. Expression of enzymes involved in Cer generation, such as neutral sphingomyelinase (SMase), acid sphingomyelinase (ASMase), serine-palmitoyl transferase, and Cer hydrolysis ceramidase, are elevated in obese adipose tissues. Hyperinsulinemia and elevated TNF-α associated with obesity contribute to increased adipose neutral SMase (NMSase), ASMase, and serine-palmitoyltransferase (SPT) mRNA (37, 150, 154). The decrease in the total adipose SM and Cer, and an increase in Sph were noted in obese mice. In contrast to adipose tissue, plasma levels of total SM, Cer, Sph, and S1P were elevated in ob/ob mice (37, 150, 154).

Phospholipid (PLs) and SLs represent half of the human plasma lipidome, with a marked diversity among humans (38, 141, 145). Portal and systemic PL profiling defined the NASH signature in morbid obesity (9, 10, 39). Lipidomic analysis showed a reduction of PC in the steatotic liver; however, PC homeostasis is an important factor for alcoholic steatohepatitis (ASH)/NASH. Patients with hepatic steatosis have 25% less PC in the liver compared with normal subjects (40, 138, 142). Phosphatidylserine is lower while phosphatidylethanolamine is diminished (41, 47, 49). Accumulation of triacylglycerols (TAG), diacylglycerols (DAG), and free cholesterol; elevated lyso-glycerophosphocholines (LPC); and a shift toward more (TAG), diacylglycerols (DAG), and free cholesterol; elevated lyso-glycerophosphocholines (LPC); and a shift toward more (TAG), diacylglycerols (DAG), and free cholesterol; elevated lyso-glycerophosphocholines (LPC); and a shift toward more TAG, diacylglycerols (DAG), and free cholesterol; elevated lyso-glycerophosphocholines (LPC); and a shift toward more structural lipids have been described in NASH (40, 138, 142). Increased concentration of several glycerophospholipid structures is more consistent in systemic circulation of NASH patients (9, 10, 39). In morbid obesity patients stratified for NASH, the PL composition in the portal circulation differs, in which they indicate...
selective alterations in lipid input to the liver. Fewer lipid species are impacted in the portal circulation compared with the changes that dominate the systemic blood (42, 139, 143). PL determines the NASH signature, and changes in PG have been shown, indicating that phosphatidylglycerols are relevant to NASH. Variation in circulatory TAG profiles defined phenotypic heterogeneity in PNPLA3 (patatin like phospholipase domain containing 3) or obesity-associated NASH (43, 78, 82).

The NASH-associated SL signature is also dependent on the SL structure (35). The SM levels are normal, while the mono-unsaturated Cers and hexosyl Cers are reduced in NASH. Sixteen of the 20 fatty acid species measured in the total lipid fraction were elevated, whereas α-linolenic acid was diminished, and medium-chain saturated fatty acids were also decreased. Plasmalogens 18:0 and 18:1 species were increased in the steatotic liver (41, 47, 49). SM and a short-chain Cer decrease in the portal circulation of NASH subjects (42, 139, 143), while PE and PG were increased in the portal circulation. The elevated portal PG and PE in NASH could be linked to altered gut microbiota, representing circulating microbial products derived from bacterial translocation (42, 139, 143). Decreased plasma essential polyunsaturated fatty acids (PUFAs), increased monounsaturated fatty acids (MUFA), and lipoxygenase metabolites were noted in NASH (42, 139, 143). An association of adipose fat cell size with NASH in the network reflects increased portal release of inflammatory mediators by hypertrophied fat cells (42, 139, 143). Postsurgery weight loss in NASH improves the level of liver enzymes and lipids, but most of the PG and Cer species remained elevated; meanwhile weight loss partially attenuated NASH-related alterations in systemic PL profiles 1 year after surgery (42, 139, 143). Analysis of lipids from the hepatic portal system at the time of surgery revealed that PG and PE classes were increased in NASH subjects; moreover, few alterations were noted in adipose tissue lipid efflux (9, 10, 39).

Serum SLs are dysregulated in patients with several chronic liver diseases, suggesting their role in liver damage (44, 62, 66). Disruptions of SL metabolism were described in hepatitis B (HBV) and C (HCV) infection, including increased expression of genes that is involved in the metabolism and transport of SLs, phospholipids, and fatty acids (33, 35, 45–47, 165, 169, 180, 184), C18DHC, C24Cer, SPH, and sphinganine were significantly linked with the level of liver fibrosis (62, 44, 66). However, a high correlation between variations in serum SL levels and responsiveness to antiviral therapy was shown. Both HCV and NAFLD induce upregulation of serum ASMase, which appears as a biomarker for these disorders (48, 63, 67).

Serum SLs is correlated with the degree of severity in insulin resistance alongside with serum triglyceride and cholesterol levels (44, 62, 66). Cer affects hepatocellular susceptibility to various stimuli, regulation of viral replication, and trafficking (6, 7, 49–51, 108, 112, 175, 179). Myriocin, an inhibitor of de novo Cer synthesis, inhibited HBV and HCV replication (52, 167, 171).

Alcohol-related liver disease (ALD) is also associated with altered Cer profiles, steatohepatitis, insulin resistance, disruptions in lipid metabolism in hepatocytes, increased proinflammatory cytokines, dysregulated lipid metabolism, ER, and oxidative stress (53, 169, 173). Altered lipid metabolism promotes Cer accumulation and oxidative stress, and these can result in a “lipotoxic state” in which there is activation of ER-stress pathways, inflammation, and insulin resistance (54, 143, 147). Liver fibrosis is linked to upregulation of Cer and ER stress-related genes. Similarly, these genes are associated with mitochondria enlargement, disruption of ER structure, lipid peroxidation, DNA damage, increased inflammatory cytokines, and impaired insulin receptor signaling. Altered Cer profiles with higher level of C14 and C18 along with reduced C16 species were noted in ethanol-exposed livers (54, 143, 147). The Cer inhibitor myriocin reduces the severity of ASH by decreasing the abundance and size of lipid droplets and mitochondria and limiting inflammation, thereby improving the architecture of the ER. Myriocin-mediated reductions in hepatic Cer levels enhance insulin signaling through the insulin receptor with a reduced hepatic oxidative stress and modulated ER stress (53, 169, 173).

**SL structure determines insulin signaling and liver damage.** The SL-acyl chain composition of the liver regulates signaling by modifying insulin receptor translocation into membrane microdomains (35, 135, 139). Long-chain (C16–C20) and very-long-chain (C22–C24) Cers exert opposing effects on cell proliferation, and also on plasma membrane fluidity (55–57, 69, 70, 73, 74, 147, 151). Mice with deleted Cer synthesis 2 (CerS2) preferentially synthesize very-long-chain Cers (C22–C24) and exhibit a compensatory increase in C16 and sphinganine levels in the liver, which alters glucose metabolism, indicating the type of GSLs can determine the degree of insulin resistance (58, 137, 141). Ablation of very-long-chain Cers is associated with induction of hepatic insulin resistance (26, 32, 34). CerS2-null mice are unable to synthesize very-long-acyl-chain (C22–C24) Cers. However, these mice suffer from glucose intolerance despite normal pancreatic insulin secretion (35, 135, 139). The lack of insulin receptor phosphorylation in the liver of this model was associated with an inability to translocate into detergent-resistant membranes. Plasma Cers were elevated in obese subjects with Type 2 diabetes and correlated with the severity of insulin resistance (59, 71, 75). Type 2 diabetic subjects had higher concentrations of C18:0, C20:0, C24:1, and total Cer. Plasma TNF-α concentrations were increased in Type 2 diabetic subjects and correlated with increased C18:1 and C18:0 Cer subspecies.

The synthesis of SLs with very long acyl chains is important for liver homeostasis (58, 137, 141). Cer is a regulator of hepatocellular apoptosis and is one of the factors contributing to the activation of HSCs. An increase in long-chain Cers and a decrease in very-long-chain Cers promote apoptosis. In Cer synthesis 2 knockout mice, ablation of very-long-chain (C22–C24) Cers was associated with severe hepatopathy and increased hepatocyte apoptosis (58, 60, 64, 68, 137, 141). Elevated C16-Cer and sphinganine levels were shown in these mice. Formation of nodules for regenerative hepatocellular hyperplasia and noninvasive hepatocellular carcinoma (HCC) were specified, and upregulation of genes having a connection with cell cycle regulation, protein transport, cell-cell interactions and apoptosis and downregulation of genes associated with intermediary metabolism, such as lipid and steroid metabolism, adipocyte signaling, and amino acid metabolism, have also been noted (64, 137, 58, 60, 68, 141).

**Role of ASMase in NASH.** A significant upregulation of acidic SMase was noted in the serum of patients with chronic liver disease compared with healthy individuals. In addition, the accumulation of various (dihydro-) Cer species was iden-
tified in the serum of patients with NAFLD and was also correlated significantly with cholesterol. SPH was upregulated in chronic liver disease; however, there was no major correlation with the markers of hepatic injury (48, 63, 67). Methionine is primarily metabolized in the liver, and its clearance is delayed in cirrhosis (61, 140, 144). Disrupted methionine metabolism, exemplified by decreased S-adenosyl-L-methionine (SAM) and/or increased S-adenosylhomocysteine (SAH) and homocysteine (Hcy) levels, and PC depletion were described in steatohepatitis (62, 112, 116). SAM is essential in liver physiology, because of its functions as a methyl donor and glutathione (GSH) precursor. Moreover, excess SAM directs phosphatidylethanolamine toward phosphatidylcholine and triglyceride synthesis (63, 110, 114).

Activation of ASMase in steatohepatitis is induced by TNF-α, reactive oxygen species, and oxidative stress (66, 64, 70). It is used in the regulation of steatosis, fibrosis, lipotoxicity, ER stress, autophagy, and lysosomal membrane permeabilization, contributing to ASH and NASH. ASMase modulates alterations of the methionine cycle and phosphatidylcholine homeostasis and regulates methylation reactions, antioxidant defense, and membrane integrity, all of which are relevant to the pathogenesis of NASH (28, 53, 57). It regulates HSC activation and liver fibrogenesis (65, 121, 125). Amtiriptiline, an ASMase inhibitor, reduces hepatic fibrosis in mice (66, 142, 146). Imipramine blocks ethanol-induced ASMase activation and Cer generation, ameliorating hepatic steatosis (67, 98, 102). ASMase-deficient mice are resistant to alcohol-induced lipogenesis, macrosteatosis, LPS sensitization, and concanavalin A-mediated liver injury (48, 50, 68). ASMase is overexpressed in the adipose tissue of ob/ob mice fed with methionine- and choline-deficient (MCD) diet, in the liver and serum samples from patients with NASH (65, 121, 125). An improvement in LDLr−/−/ASMase−/− mice were associated with a paradoxical increase in hepatic Cer levels and de novo Cer synthesis due to the increased SPT expression (69, 131, 135). ASMase deficiency interrupted the expression of ER stress markers after alcohol or HFD feeding (48, 50, 68). Although the chemotherapeutic agent cisplatin-activating ASMase leads to a exogenous delivery of D-e-C16-Cer, dihydro-C16-Cer does not result in changes in cell morphology and actin cytoskeleton in breast cancer (70, 183, 187). ASMase-induced Cer generation downregulates MAT1A mRNA expression, and decreases in MATI/III protein levels are induced by TNF-α, resulting in SAM depletion and TNF-mediated liver failure (71, 107, 111). ASMase triggers the accumulation of GSLs, in particular ganglioside GD3 (51, 54, 72). In addition to ASMase activation, MCD or methionine-deficient (MD) diets deplete PC levels, in which the PC/PE ratio in liver homogenates and mitochondria decreases (29, 31, 73), since Cer can be deacylated by ceramidases to SPH.

Role for GSLs in the progression from NASH to liver cancer. SLs are localized in selective membrane domains that are linked to cell signaling and regulate cell death pathways. They affect subcellular organelles including the mitochondria, ER, and lysosomes to mediate apoptosis, ER stress, autophagy, and necroptosis. Thus they are of relevance to cancerous processes (54). The metabolic syndrome and NASH are risk factor for the development of HCC (117, 164). GSLs were suggested as a potential connection between them. Oral administration of GC was shown to suppress HCC growth in vivo (190). Tumor-associated carbohydrate antigens including GSLs are expressed on the tumor cell surface. Fucosylated neutral GSLs in HCC are highly expressed on tumor tissues (187). Nanoliposomal C6-Cer is an autophagy inducer that enhances apoptotic cell death in cancer (3). Cinobufotalin, a bufadienolide isolated from toad venom, inhibited HCC cell growth while inducing cell apoptosis. Treatment was associated with inhibition of sphingosine kinase 1 (SphK1) activities along with the induction of pro apoptotic Cer production (34). Cer synthase-1 small hairpin RNA (shRNA)-depletion inhibited cinobufotalin-induced Cer production and HCC cell apoptosis. SphK1 inhibitor II (SKI-II) increased cellular Cer level and promoted HCC cell apoptosis. The glucosylceramide synthase (GCS) inhibitor 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) facilitated cinobufotalin-induced Cer production and HCC apoptosis (34). Alterations of serum core fucosylation, outer-arm fucosylation, increased sialylation, and gycan branching were described in patients with HCC. Similar findings have been found directly in HCC tissue, suggesting that these glycans changes may play a role in tumor formation and development (115).

Generally, these data support a pathogenetic role for SLs in insulin resistance, diabetes liver steatosis, and degree of liver damage. These data also imply that any change in one of the constituents of these pathways is likely to alter the levels of other upstream and downstream elements, thereby affecting several cellular functions, which may underlie major mechanisms of NASH and diabetes.

Patients with Gaucher’s Disease Before and After Enzyme Replacement Therapy and Alteration of the Immune System

GD is the most common lysosomal storage disorder and has an autosomal recessive pattern of inheritance. GD is caused by defective activity of acid-β-glucosidase (GlcCerase), resulting in the accumulation of GC in macrophages and the deposition of GC in multiple organ systems (81–83, 87, 133, 137, 170, 174). Enzyme deficiency leads to the accumulation of GC in lysosomes, which is central to disease pathogenesis involving visceral organs, bone, or brain. GSLs are endocytosed to the Golgi apparatus in normal cells, although they are mistargeted to lysosomes in GD (84, 158, 162).

Secondary biochemical pathways regulating levels of PL metabolism are also altered in GD (81, 170, 174). In the peripheral tissues, the cerebellum and cerebrospinal fluid of patients with GD, an increase in total GSLs was observed (61, 65, 85). A relative increase in GD3 was noted in the central nervous tissue. Analysis of the GC structure revealed a prevalence of stearic acid in the central nervous system, and palmitic acid in the peripheral tissues (61, 65, 85). Treatment of macrophages with the GlcCerase inhibitor conduritol-B-epoxide (CBE) observed PC and [methyl-14C]choline. These changes correlated with the accumulation of [1H1]GlcCer (81, 170, 174). However, a negative correlation was shown between plasmalogens species, GC, Cer, the GC/Cer ratio, glucosylphingosine, and malonyldialdehyde, which is significant for the C16:0 species. A positive correlation with total antioxidant status was recorded, indicating an increased lipid peroxidation and reduced total antioxidant status in GD (86, 122, 126).

GD is related to cellular abnormalities that were suggested to facilitate the growth of malignant clones (13, 14, 87). These
include disturbances in natural killer T (NKT) cells, GC deposition, chronic antigenic stimulation, increased free radical production, impaired antigen presentation, reduced intracellular Cer levels, and disturbed autophagy (13, 87, 14). Following enzyme replacement therapy (ERT), GC levels decrease by changing the GSL balance in these patients.

Much has been learned about the role of GSLs in the immune system from patients with GD (79, 83, 88). These patients have altered humoral and cellular immune profiles, including altered NKT cell numbers and function. Data from GD suggest an effect of GC on the cell membrane; hence, some patients have increased red blood cell aggregation due to changes in the properties of the cell membrane (1, 2, 89). Studies of these patients have implied that GC is involved in NKT cell regulation (90, 105, 109). Patients with GD showed upregulation of CD1d and major histocompatibility complex (MHC)-II expression by monocytes, which are associated with inflammation (14, 15, 91). ERT decreases MHC-II expression in GD, which correlates with chitotriosidase activity and is a marker of inflamed macrophages (14, 15, 91). Treatment with CBE, an irreversible inhibitor of glucerase activity, of monocytes either from GD patients or healthy subjects, leads to increased surface expression of the lipid-binding molecule CD1d (15, 92, 16).

Monocytes from GD patients highly express MHC-II. ERT induced a decrease in MHC-II expression and partial correction of the lymphocyte imbalance (15, 16, 92). Monocytes derived from ERT-treated GD displayed increased levels of CD1d and MHC-II. Alteration of CD4+, CD8+, and Vo24+ T cells were also described following ERT. Patients with GD exhibit a decreased number of CD4+ helper lymphocytes and increased frequencies of CD8+ suppressor lymphocytes, resulting in a significantly decreased CD4/CD8 cell ratio. Impairment of regulatory T cells, along with increased IFN-γ-producing CD4+ and CD8+ T cells, was noted, indicating a TH-1 polarization pattern (93, 161, 165). These patients manifested decreased percentages and absolute numbers of CD4+CD25dim, CD4+CD25high, and CD4+CD25highFoxP3+ cells, respectively (93, 161, 165).

GC is important for the activation of antigen-presenting cells (APCs), induction of Th1 and Th17 responses, and neutrophil (PMN) recruitment (94, 134, 138). Increases in several immune cell populations were identified in the Gba1 mouse model (D409V/null; 9V/null) of GD, including APCs, dendritic cells (DCs), PMNs, and CD4+ T cells. Macrophages, DCs, PMNs, and T cells from these mice showed excess GC as a potential basis for activation of Th1 (IFN-γ, IL-12, TNF-α) and Th17 (IL-17A/F) cytokine production (94, 134, 138). Increased level of chemokines, the corresponding monocytes, DCs, PMNs, T cells, and B cells were also identified in these mice, which may underlie the pathological trafficking of immune cells to sites of inflammation in GD (83, 133, 137). Enhanced chemotaxis of bone marrow-derived macrophages, DC, and T and B lymphocytes was found in the lung, spleen, and liver (83, 133, 137).

Patients with GD exhibit quantitative defects in their DCs as demonstrated by decreased circulating DC precursors in both myeloid and plasmacytoid types (95, 118, 122). Numbers of myeloid (mDC) and plasmacytoid (pDC) DCs were decreased. pDC from GD showed a decrease in IFN-α production after TLR-9 stimulation compared with controls, in which ERT restored their function (27, 28, 96). Both myeloid and plasmacytoid DC and the yield of the monocyte-derived DC were obtained due to a decrease in GM-CSF and IL-4 stimulation. CD34+ cell differentiation in the presence of GM-CSF, SCF, TNF-α, and IL-4 into mature DCs was normal (95, 118, 122).

The cellular alterations in GD produce a proinflammatory milieu mediated by proresorptive cytokines, such as TNF-α, leading to bone destruction through enhancement of monocyte differentiation to osteoclasts and osteoclast resorption activity (97, 124, 128). GC modulates endolysosomal pH in lymphocytes, suggesting that this mechanism is disrupted in GD (84, 158, 162). Substrate reduction therapy utilizes inhibitors of GC synthase increases cholesterol, triacylglycerol, and the endolysosomal pH in macrophages. GC modulation appears to be specific because glucosylsphingosine reversed the effects of GlcCer depletion, unlike galactosylsphingosine. Accumulation of SL in monocytes can lead to regulatory T cell (Treg) imbalances in GD (15, 92, 16). The accumulation of GC may result to inappropriate cross talk by immune cells and the perpetuation of chronic inflammation in these patients (14, 15, 91). Accumulated GC in the reticuloendothelial organs leads to chronic antigenic stimulation of the immune system, resulting in polyclonal hypergammaglobulinemia, and mononuclear populations of lymphocytes and plasma cells may arise from this proliferative state (17, 18, 98, 99, 146, 150). GC increases the risk of multiple myeloma and T cell dysfunction as a result of high levels of ferritin and/or other factors released by monocytes. However, cases of type I GD coexisting with relatively low serum immunoglobulins, impaired antibody production, and recurrent bacterial infections have been described (82, 83, 87). All of these underscore that ineffective T cell control exposes patients to inflammatory reactions, poor immune responses against infectious agents, and impaired immune surveillance associated with an increased risk of neoplasm (93, 95, 118, 122, 161, 165). A contrasting point of view suggests that GC accumulation may result to several evolutionary advantages (79, 88, 83), as secondary biochemical pathways upstream and downstream of GC that regulate phospholipid metabolism are affected in GD. Carriers of GD might not have an increased risk in other diseases (100, 136, 140). Resistance to tuberculosis (88, 92, 101), superior intelligence (37, 39, 102), and increased fertility (103, 162, 166) are other possible advantages associated with GC.

The overall estimated life expectancy for patients with GD type 1 (GD1) is 9 years, which is less than that for a reference population (104, 177, 181). The estimated life expectancy for splenectomized GD is lower than that for nonsplenectomized patients, and the causes of death linked to splenectomy were malignancies, cardiovascular disease, and cerebrovascular disease (104, 105, 114, 118, 177, 181). In a retrospective study, the incidence of malignancies in a cohort of patients with GD1 showed no statistically significant difference relative to age-matched subjects. Four percent of GD patients developed cancers. The most common malignancies were lymphoma, myelodysplastic syndrome, and multiple myeloma. In patients with GD, there was no increased risk of malignancy in the International Registry Report of Gaucher (ICGG) with the exception of multiple myeloma (106, 193, 197). In a registry of 2,742 GD patients, although the incidence of multiple myeloma was increased, there was no increased risk of breast,
indicated that GD is associated with hypermetabolism. In untreated GD, the prevalence of obesity and diabetes is lower than in the general population (94, 98, 130).

Overweight and metabolic syndrome are common in patients with GD type I on ERT (45, 47, 132). In a cross-sectional study of patients with GD type I (n = 15), seven were overweight, whereas five of the eight patients presenting with metabolic syndrome had insulin resistance. Leptin levels were inversely correlated with LDL-cholesterol and directly correlated with BMI, waist circumference, enzyme dosage, triglycerides, insulin, and homeostasis model assessment (HOMA) of insulin resistance. Ghrelin and adiponectin levels correlated with each other; they were inversely correlated with BMI, waist circumference, and triglyceride levels, in which they were directly correlated with HDL-cholesterol (HDL-c) (45, 47, 132). HDL-c levels are abnormally low in type I GD. However, the low HDL-c levels do not lead to premature atherosclerosis, as assessed by carotid artery intima-media thickness measurement in GD (40, 42, 133). Leptin is strongly associated with insulin and the HOMA index and was suggested as a biomarker to assess early evidence of insulin resistance in patients with GD (45, 47, 132).

The insulin resistance reported in patients with GD is associated with altered SL metabolism (49, 52, 120). Cer and more complex SLs are the building blocks of lipid rafts in membrane microdomains. Lipid rafts regulate lipid and protein interactions, which are required for insulin signaling. The loss of insulin receptor from lipid rafts is due to the accumulation of GM3 (81, 85, 134). Complex GSLs, such as ganglioside GM3, surround the insulin receptor in the raft membrane compartment and modulate signaling through this receptor (95, 99, 129). GM3 concentrations, for which GC is the precursor, are elevated in plasma and several cell types in GD (58, 62, 128). Increased levels of GM3 in rafts impair insulin signaling, resulting in insulin resistance. The ganglioside GM3 is a negative regulator of insulin sensitivity (58, 62, 128). In animal models of GD, lipid composition is altered, contributing to impaired insulin signaling (49, 52, 120), whereas, in fibroblasts from GD patients, decreased degradation and increased anabolism of GC were detected. Plasma Cer concentrations in untreated GD patients were lower than in controls, although the GC and GM3 levels were elevated. Plasma GM3 concentrations correlated with plasma chitotriosidase activity, overall disease severity, and hepatomegaly (58, 62, 128). Lipid rafts are affected in GD in a way that controls Akt signaling (49, 52, 120, 135, 159, 163). Akt phosphorylation was impaired in a Gaucher cell model.

Macrophage activation leading to inflammation is another potential mechanism connecting GD with insulin resistance (136, 156, 160). Both IL-6 and TNF-α are elevated in GD during insulin resistance. Macrophages with anti-inflammatory properties are considered M2, whereas M1 indicates classically activated macrophages that secrete proinflammatory cytokines. The effect of GC accumulation of macrophages in GD may contribute to their phenotypic features. This type of lipotoxicity was suggested to be the nexus between GD and insulin resistance (49, 52, 120).

ERT leads to a decrease in resting energy expenditure. Long-term ERT induces a larger than average weight gain, resulting in a similar prevalence of obesity in ERT-treated patients and the general population. Insulin resistance was also described in nonoverweight ERT-treated patients (131, 171, 175).
In adults, changes in energy metabolism remaining during ERT, insulin resistance, and weight gain were described in association with ERT (46, 48, 137). The prevalence of Type 2 diabetes increases significantly during treatment with ERT, resulting in a comparable prevalence of Type 2 diabetes in treating patients in the general population (94, 98, 130). In a recent literature search, it was found that GD patients showed low weight and height before ERT. ERT normalized the growth of children and adolescents with GD type I (46, 48, 137). ERT can also induce insulin resistance. It is unclear either this is due to reduction in GC level or secondary SI alterations, such as transient increases in Cer as excess GC passes through the catabolic pathways (49, 52, 120). The reduction of SL synthesis by ERT may alter both lipotoxicity and the inflammatory state induced by the lipotoxicity.

Generally, these data recommend a protective effect for GC on inflammatory cells that may explain the pathogenesis of diabetes and cardiovascular diseases (39, 41, 138). The steady state in the GSLs achieved under ERT may not be equal in all patients because not every GD patient has insulin resistance nor does everyone developed diabetes under ERT. Therefore, other factors may contribute to the development of these conditions.

Modulation of the SL Pathways and Use of Their Constituents as Secondary Messenger Molecules for the Treatment of Diabetes, NAFLD, and NASH

SL metabolic pathways are attractive targets for novel therapies of NASH and diabetes. Blockade or induction of an enzyme in the SL pathways, or altering the quantity of one or more of the constituents of these pathways, is expected to result in a respective increase and decrease of upstream and downstream secondary GSL messenger molecules (4, 5, 31). The results described below provide further evidence that GSL metabolites play a role in mediating a link between obesity and insulin resistance. Moreover, interference with GSL biosynthesis by decreasing or increasing various compounds changes the steady state of these secondary messengers, making them novel targets for therapy for both NASH and NASH. Much of the conflicting results described are due to the development of different GSL steady states under various disease conditions as well as host-determined or environmental confounding factors. Most studies have focused on one compound of the SL pathways rather than on the general new steady state, which involves an increase or decrease of many GSLs upstream or downstream of the target compound. In addition, plasma levels and tissue levels of GSLs do not correspond with each other, and different signatures have not been monitored in all studies.

Altered Cer Levels Affect Liver Steatosis and Insulin Resistance

SL regulates key processes involved in steatosis and insulin resistance, such as ER stress and autophagy. Insulin-mediated peripheral glucose uptake was reduced after prolonged fasting associated with increased intramuscular Cer concentrations and decreased phosphorylation of AKT (160, 139, 164). However, glucocorticoid treatment impaired whole-body insulin sensitivity in healthy men without concomitant changes in muscle Cer, GM3, or mitochondrial function (26, 140, 27). Environmental stress and chronic inflammation stimulates Cer signaling and induces cellular senescence with proinflammatory responses (141, 148, 152). An increase in liver Cer secretion protects it from harmful intracellular accumulation (142, 176, 180). Plasma Cer levels were increased during lipid infusion in humans and rats as well as in obese, insulin-resistant mice (142, 176, 180). Balanced diet deficiency in both methionine (MD) and choline (CD) are the common models of steatosis, mitochondrial dysfunction, hepatocellular injury, oxidative stress, inflammation, and fibrosis (113, 143, 117). The MD diet is associated with weight loss, hepatocellular injury, oxidative stress, inflammation, and fibrosis, whereas CD results in steatosis (29, 31, 73). MCD- or MD-mediated mitochondrial SAM and GSH depletion is due to the decreased mitochondrial membrane fluidity associated with a lower phosphatidylcholine/phosphatidylethanolamine ratio. MCD and MD resulted in increased Cer levels by ASMase, whereas GSH ethylester or SAM therapy restored mitochondrial GSH and ameliorated the liver injury in these mice (29, 31, 73).

Increased levels of perilipin 2 (Plin2) have been associated with the development of steatosis, glucose intolerance, and Cer accumulation in ALD. Plin2KO mice chronically fed alcohol were protected from the hepatic Cer accumulation, steatosis, and glucose intolerance, suggesting a critical pathogenic role for Plin2 in ALD and further supporting the potential role of increased Cer in steatosis and insulin resistance (31, 33, 144).

Dysregulation of lipid metabolism increased the accumulation of hepatic Cer and worsen insulin resistance, which affected liver function. Insulin and insulin growth factor (IGF) stimulate aspartyl-asparaginyl-β-hydroxylase (89, 93, 145). Aspartyl-asparaginyl-β-hydroxylase (AAH) mediates its effect by activating Notch. Insulin/IGF signaling, AAH, and Notch are inhibited in ALD (99, 103, 146). Chronic ethanol-fed rats had shown an increased Cer levels and steatohepatitis, with impairments in insulin receptor signaling, insulin receptor substrate, and Akt. Neurodegeneration and brain insulin resistance are linked with increased hepatic production of neurotoxic Cers that crossed the blood-brain barrier (101, 147, 105). High-fat diet (HFD)-induced obesity with T2DM causes mild neurodegeneration (101, 105, 147). HFD feeding gradually increases the body weight while decreasing brain weight, thereby increasing Cer synthase, serine palmitoyl transferase, and SMase expression in the liver except in the brain. Temporal lobe levels of ubiquitin and 4-hydroxynonenal were increased; however, tau, β-actin, and choline acetyltransferase levels were equally decreased (101, 105, 147).

Low levels of hepatic PC play a role in the pathogenesis of NASH (145, 148, 149). CTP, a phosphocholine cytidylyltransferase (CT), is a key regulatory enzyme in the CDP-choline pathway for PC biosynthesis. Liver-specific elimination of CTβ decreases very low-density lipoprotein secretion, reduces lipid efflux from the liver, and induces steatosis. In HFD, these mice developed NASH along with an increase in Cer and a decrease in PC content. Daily administration of CDP-choline or betaine did not prevent the development of NASH, implying that normalizing the amount of hepatic PC is not sufficient to prevent NASH (127, 131, 149). Based on the notion that increased Cer levels may be deleterious, inhibition of de novo Cer synthesis with myriocin affected lipid metabolism in the liver of rats with streptozotocin-induced Type 1 diabetes (91, 95, 150). Myriocin abrogated many of the adverse effects of...
ethanol, including hepatic Cer accumulation, steatohepatitis, and impairments of insulin signaling (99, 103, 146).

Although a reduction in Cer levels was suggested to alleviate steatosis, an opposing effect has been described in several studies. Cer-mediated suppression of peroxisome proliferator-activated receptor (PPAR) γ2 reduces the expression of CD36 and Fsp27, and also reduces liver steatosis (97, 151, 101). SMS2 inhibition in the liver could diminish liver steatosis (97, 151, 101). In a hyperinsulinemic-euglycemic clamp clinical trial in obese adults, a negative correlation was shown between insulin-mediated suppression of hepatic glucose production and intrahepatic diacylglycerol; however, it didn’t occur with intrahepatic Cer or acylcarnitine (102, 152, 106).

A causal link between Cer synthesis and ER stress were described in lipotoxicity. Palmitic acid (PA) induces hepatocyte apoptosis and fuels de novo Cer synthesis in the ER. Myristic acid (MA) is a free fatty acid highly abundant in copra/palmist oils and is a predictor of NASH that stimulates Cer synthesis. MA is not lipotoxic but potentiated PA-mediated lipaopoptosis, ER stress, caspase-3 activation and cytochrome c, which is released in primary mouse hepatocytes (111). Treatment with myriocin inhibits Cer synthesis and tauroursodeoxycholic acid to prevent ER stress, thus ameliorating apoptosis. Myriocin treatment hinders lipodystrophy and hepatosplenomegaly and then increases liver Cer content, ER stress, liver damage, inflammation, and fibrosis in mice fed a diet enriched in PA plus MA (111).

Collectively, these data support an attempt to alter Cer levels as a means for the treatment of NAFLD and insulin resistance.

Altered GSL and SM Levels Affect Liver Steatosis and Insulin Resistance

Pharmacological inhibition of GSL synthesis improves insulin sensitivity in rodents with insulin resistance (32, 93, 97). Meanwhile partial GSL reduction is a therapeutic venue for obesity-induced insulin resistance and Type 2 diabetes. In vivo, inhibition of GSL biosynthesis in animals ameliorated insulin resistance, and this effect was accompanied by improved glycemic control and decreased liver steatosis in obese mice (84, 88, 154). Pharmacological GSL depletions altered hepatic secretory function, whereas SM inhibits intestinal cholesterol absorption. Dietary egg SM has liver lipid-lowering properties in mice maintained on an obeseogenic diet (35, 37, 155). Mice supplemented with SM had significantly increased fecal lipid and cholesterol output and reduced hepatic cholesterol levels. SM synthase (SMS) is a substrate to produce SM and is at the crossroads of SL biosynthesis (97, 151, 101). SMS has two isoforms; SMS1 and SMS2. SMS2 is the major isoform in the liver. SMS2 liver-specific transgenic livers have lower Cer and higher SM, whereas SMS2 knockout livers have higher Cer and lower SM. SMS2 overexpression promoted fatty acid uptake and liver steatosis, whereas SMS2 deficiency had an opposite effect.

Reduction in hepatic lipid and plasma non-HDL cholesterol were noted in Zucker fatty rats fed with SM of animal origin and GC of plant origin. Both SM and GC diets decreased plasma insulin levels, and the GC diet increased the plasma adiponectin level. These effects were associated with increased expression of adiponectin receptor 2, PPARα, and pyruvate dehydrogenase kinase 4 and with a decreased in the expression of stearyl CoA desaturase (156, 182, 186).

Dietary sea cucumber cerebroside (SCC) extracted from Acaudina molpadioides attenuated hepatic steatosis via inhibition of hepatic lipogenic gene expression and enzyme activity, and enhancement of triglyceride secretion from the liver (157, 184, 188). SCC inhibited hepatic lipogenic enzymes, including fatty acid synthase, malic enzyme (ME), and glucose-6-phosphatedehydrogenase (G6PDH). Gene expression of FAS, ME, G6PDH, and sterol-regulatory element binding protein (SREBP)-1c was reduced. These effects were also linked with altered hepatic MUFA and SL content, affecting the expression of enzymes involved in MUFA and SL synthesis. Dietary docosahexaenoic acid (DHA, 22:6,n-3) reversed Western diet-induced NASH in LDLR−/− mice (43, 45, 158). DHA protected against Western diet-induced NASH via regulation of hepatic SFA, MUFA, PUFA, SM, PUFA-derived oxidized lipids, and 5-lactosylglutathione (43, 158, 45).

Manipulation of SL synthetic pathways is also a method of treatment of low-HDL dyslipidemia and atherosclerosis (159, 179, 183). SREBPs are transcription factors of lipid metabolism that regulate more than 30 genes of cholesterol, fatty acid, and PL synthetic enzymes (159, 179, 183). GSLs regulate SREBP-dependent lipid synthesis, ATP-binding cassette protein ABCA1, and ABCG1-mediated lipid efflux, which regulate cellular lipid homeostasis. SL synthesis activates SREBPs. SREBPs are downstream of SL synthesis and do not regulate the activity of SL synthetic enzymes. Cells that cannot synthesize SLs fail to increase SREBP in response to lipid depletion (159, 179, 183). Cer decreases SREBP by inhibiting SL synthesis (159, 179, 183). There is an inverse relationship between de novo SL synthesis and cholesterol efflux. SLs regulate cholesterol efflux receptors ABCA1 and ABCG1, which are the major regulators of plasma HDL (159, 179, 183). Inhibition of de novo SL synthesis increases ABCA1-mediated cholesterol efflux independent of SM, increasing antiatherogenic lipoproteins and decreasing atherosclerosis in mice (159, 179, 183). Breakdown of plasma membrane SM by TNF-α inhibits glucose metabolism and insulin signaling in muscle and fat cells (160, 173, 177).

Overall, these data support the notion of GSLs and SM as potential targets for the treatment of fatty liver disease and NAFLD.

Glucosylceramide Synthase Inhibitors and Other Inhibitors of GSLs

An inhibitor of GCS, an enzyme that catalyzes the conversion of Cer to GSLs, improved insulin sensitivity and T2DM (5, 6, 154, 158, 161, 162). GENZ-123346 is a specific inhibitor of GCS, an enzyme that catalyzes an important step in the conversion of Cer to GSLs. Genz-123346 alleviates insulin resistance, reversing hyperinsulinemia and improving glucose tolerance in mice with diet-induced insulin resistance and NASH in animal models (185, 186, 163, 164, 189, 190).

The iminosugar derivative N-(5’- adamantane-1’yl-methoxy)-pentyl-1-deoxyojirimycin (AMP-DNM) is a small-molecule inhibitor of GCS (5, 6, 161). In adipocytes, AMP-DNM counteracted TNF-α-induced abnormalities in GSL concentrations, reversing the abnormalities in insulin signaling transduction in vitro. In ob/ob mice, HFD mice, and ZDF rats,
AMP-DNM normalized the elevated tissue of GC and GM3 levels, subsequently lowering the circulating glucose level, hence improving oral glucose tolerance and insulin sensitivity in muscle and liver (5, 6, 161). AMP-DNM resulted in a 70% reduction of GSLs in the liver hepatoma cell line HepG2 (20, 21, 165). The effect was associated with a significant up- or down-regulation of target genes for the transcription factors SREBP1 or SREBP2, which activate genes in the sterol biosynthesis pathway. The associated increase in cholesterol production was as a result of the activation of the cholesterol biosynthesis pathway (20, 21, 165). AMP-DNM treatment decreased plasma level of triglycerides and cholesterol, while neutral sterol, biliary lipids, and bile flow excretion is increased twofold (22, 23, 166). Clinically, treatment led to decreased plasma cholesterol and triglyceride levels. Treatment of ob/ob mice with AMP-DNM restored insulin signaling in the liver, corrected blood glucose levels, and decreased insulin concentrations (21, 22, 167). The result was related with the normalization of the expression of SREBP1c target genes involved in fatty acid synthesis. The liver/body weight ratio decreases with the reversal of hepatic steatosis and with an increase in inflammatory markers. AMP-DNM treatment corrected the gene expression profile of ob/ob mouse livers (21, 22, 167). A reduction of GSL biosynthesis in the adipose tissue of ob/ob mice restored insulin signaling in isolated ex vivo insulin-stimulated adipocytes (168, 172, 176). The improved adipogenesis was a result of the reduced number of larger adipocytes and increased expression of PPARγ, insulin-responsive glucose transporter (GLUT)-4, and adipin (103, 107, 168, 169, 172, 176). Adiponectin gene expression and protein were increased by AMP-DNM, leading to a decreased inflammation in adipose tissue. AMP-DNM-treated LDLR−/− mice on a Western diet showed reduced liver steatosis, inflammation, and fibrosis. Induction of fatty acid β-oxidation and plasma lipids was observed, indicating that AMP-DNM revised the preexisting NASH (100, 104, 170). Initiation of treatment with AMP-DNM resulted in a rapid increase in fat oxidation, a decrease in carbohydrate oxidation, and a reduction in food intake (96, 100, 171). The effect was associated with increased plasma level of the appetite-regulating peptide YY (96, 100, 171). The 1-ido derivative of 2,1-ido-AMP-DNM, a selective inhibitor of GSL synthesis, lowered visceral GSLs in ob/ob mice and ZDF rats, with no inhibition of sucrase activity or sucrose assimilation being less effective in reducing blood glucose and HbA1c (172, 178, 182).

In contrast, inhibitors of GSL biosynthesis via inhibition of the enzyme Ugeg (UDP-glucose: Cer glucosyltransferase), that catalyze the first step of the glucosylceramide-based GSL-synthesis pathway, did not exert a similar effect (84, 88, 154). Ugeg inhibitors may exert their effect on hepatocytes independently of GSLs (157, 161, 173). A preventive effect of GSL deficiency on the development of liver steatosis after an HFD was not observed. Deletion of GSLs in hepatocytes by use of specific inhibitors did not change the quantity of bile excretion through the biliary duct; cholesterol concentrations in the liver, bile, feces, and plasma; or lipoprotein concentrations in plasma. No alterations in glucose tolerance after intraperitoneal application of glucose and insulin were observed in mutant animals. Inhibiting GCS by use of the iminosugar-based inhibitor miglustat (NB-DNJ) has been reported to increase the survival of Niemann-Pick mice. The oral administration of Genz-529468, an improved iminosugar-based inhibitor of GCS, to NPC mice in which there is an enhancement in their motor function, reduced central nervous system inflammation, and increased their longevity (128).

Overall, these data aid in the concept that GSL levels are associated with both liver steatosis and insulin resistance. The lack of consistency in the effects by different treatments may be explained by dissimilar results on many GSLs, inducing various “GSL signatures” and diverse steady states.

**SPH-1-Phosphate Receptor Modulator**

Fingolimod, FT-720, is a SPH-1-phosphate receptor modulator that interferes with SL signaling and has immune modulatory functions (18, 19, 67, 71, 175, 176). The cellular balance between Cer and SPH-1-phosphate (SIP) regulates cell growth and death. SIP receptor agonists are useful, as shown for the SL-like immunomodulatory substance FTY720 (10, 30). When phosphorylated via SPH kinase to yield FTY720, phosphate acts as an agonist of SIP receptors; hence, it displays antagonistic activity upon a prolonged presence by desensitization of the SIP receptor (77, 81, 179). FTY720 inhibits the movement of lymphocytes to the central nervous system by inducing their accumulation in lymph nodes and ameliorating multiple sclerosis (7, 8, 55, 90, 180). Treatment with FTY720 obstructed diabetes manifestation, islet infiltration, and β-cell destruction (168). Furthermore, it also led to increased gene expression of IL-1β, TNF-α, and CD8 markers in the pancreatic-draining lymph nodes, indicating immune cell activation (55, 87, 91, 183). The SpHK1/SIP pathway is also a potential target for the treatment of chronic kidney diseases (152, 156, 184). SPH-1-phosphate affects glomerulonephritis, including mesangial cell proliferation, renal inflammation, and fibrosis. Sonoprizumab is a monoclonal anti-SIP antibody that neutralizes extracellular SIP and was recommended for the treatment of glomerulonephritis (152, 156, 184). These data may have implications for its potential antifibrotic effect, which may also apply to hepatic fibrosis.

**Oral Administration of GC for the Treatment of Insulin Resistance**

Oral administration of GSLs is effective in models of acute and chronic liver diseases, including NASH (80, 84, 104, 108, 185–188, 191, 192, 195, 196). In the ob/ob model, oral administration of GC decreased liver size and hepatic fat content, coupled with an improved insulin resistance and serum triglyceride levels. An increased peripheral/liver NKT ratio, decreased proinflammatory cytokines, and increased anti-inflammatory cytokines were noted (104, 108, 187). In the Cohen diabetes-sensitive rat, a lean model of non-insulin-resistant, nutritionally induced diabetes, administration of GC, β-lactosylceramide (LC), or the combination of both, improved pancreatic and liver histology with reduced hepatic steatosis and glucose metabolism. These effects were linked with increased intrahepatic trapping of CD8+ T and NKT lymphocytes (186, 192, 196). A similar beneficial effect of a combination of GC and LC was noted in the *Psammomys obesus* high-energy diet model, and its treatment was associated with decreased liver enzymes, liver weight, hepatic fat, and improved liver histology, with improved serum cholesterol and triglyceride levels (185, 191, 195). Preliminary results support their beneficial
role in patients with NASH (189, 193). In a per-protocol analysis of 23 patients enrolled in a double-blind controlled trial, oral administration of GC decreased the hepatic fat content, as measured by MRI with improved HbA1c serum levels. The beneficial effects were related to a decrease in CD4+ and NKT cell subsets of lymphocytes (189, 193).

**Effects of FXR Agonists and Anti-LPS in NASH Are Partially Associated with Increased Cer: A Possible Connection Between GSLs and the Gut Microbiome**

The bile acid derivative 6-ethylcholenedioxycholic acid (obeticholic acid) is a potent activator of the farnesoid X nuclear receptor (FXR) that reduces liver fat and fibrosis in animal models of fatty liver disease. FXR activation attenuates LPS-induced hepatic inflammation in murine NAFLD by reducing expression of proinflammatory cytokines in macrophages (181, 185, 190). In a recent clinical trial, obeticholic acid improves the histological features of NASH (126, 130, 191). One of the underlying mechanisms for the effect of FXR agonists was associated with alterations of Cer. Inhibition of an intestinal FXR/Cer axis-mediated gut microbiota-associated NAFLD development links SLs with the microbiome, nuclear receptor signaling, and NAFLD (86, 90, 192). Mice with intestine-specific FXR disruption had reduced hepatic triglyceride accumulation in response to a HFD due to insufficient circulating Cers, which resulted from lower expression of Cer synthesis genes. The reduction of Cer levels in the ileum and serum downregulated hepatic SREBP1C and decreased de novo lipogenesis. Administration of C16:0 Cer reversed hepatic steatosis (86, 90, 192).

Oral administration of anti-LPS antibodies along with GSL-enriched adjuvant compounds was effective in ameliorating NASH and diabetes in both animal models and humans (2, 3, 120, 124, 193, 194). These effects were associated with alterations of Tregs and NKT cells, thereby supporting a potential effect of GSLs on the gut-microbiome-immune system axis.

**Summary**

Building block constituents of the SM-Cer-GSL map play a role as secondary messenger molecules in metabolic and the immune system pathways. Accumulated data from patients with GD and lipidomics data from patients with NAFLD/NASH and diabetes support their potential role in the pathogenesis of these disorders. These compounds may serve as novel therapeutic targets for NAFLD/NASH and T2DM. Preliminary human data aid in the potential use of these molecules as a means for promoting a new SL steady state that can be used to overcome several hits associated with the development of liver steatosis, inflammation, and insulin resistance.

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**AUTHOR CONTRIBUTIONS**

Y.I. conception and design of research; Y.I. analyzed data; Y.I. interpreted results of experiments; Y.I. prepared figures; Y.I. drafted manuscript; Y.I. edited and revised manuscript; Y.I. approved final version of manuscript.

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