

## Minireview

# Ceramide and Sphingosine 1-Phosphate in Liver Diseases

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**The liver is an important organ in the regulation of glucose and lipid metabolism. It is responsible for systemic energy homeostasis. When energy need exceeds the storage capacity in the liver, fatty acids are shunted into nonoxidative sphingolipid biosynthesis, which increases the level of cellular ceramides. Accumulation of ceramides alters substrate utilization from glucose to lipids, activates triglyceride storage, and results in the development of both insulin resistance and hepatosteatosis, increasing the likelihood of major metabolic diseases. Another sphingolipid metabolite, sphingosine 1-phosphate (S1P) is a bioactive signaling molecule that acts via S1P-specific G protein coupled receptors. It regulates many cellular and physiological events. Since an increase in plasma S1P is associated with obesity, it seems reasonable that recent studies have provided evidence that S1P is linked to lipid pathophysiology, including hepatosteatosis and fibrosis. Herein, we review recent findings on ceramides and S1P in obesity-mediated liver diseases and the therapeutic potential of these sphingolipid metabolites.**

**Keywords:** ceramide, fibrosis, insulin resistance, obesity, sphingosine 1-phosphate, steatosis

## INTRODUCTION

Overnutrition and consequent obesity is the major cause of metabolic dysfunction, a general term that includes insulin

resistance, cardiometabolic diseases, and non-alcoholic fatty liver disease (NAFLD) (Barr et al., 2010; Hastie et al., 2010). Overeating inhibits catabolic metabolism while activating anabolic synthetic pathways and energy storage. Obese subjects have elevated free fatty acids (FFA) in the blood plasma, and these accumulate in tissues such as the liver that do not normally contain adipose tissue (Bradbury, 2006; Unger, 2003). Abnormal accumulation of FFAs in obese people promotes the synthesis of triglycerides (TGs), the preferential energy storage form in humans.

Patients with NAFLD do not consume a significant amount of alcohol, but do accumulate TGs as lipid droplets in their liver. The prevalence of NAFLD has increased 25% to 30% over the past 20 years (Bashiardes et al., 2016), and the prevalence of other metabolic diseases, for instance, type 2 diabetes, hyperlipidemia, and cardiovascular diseases, has also increased during this time (Younossi et al., 2016). NAFLD is an umbrella term for a broad range of liver diseases ranging in severity from simple steatosis to inflammatory steatohepatitis. Simple steatosis is the mildest form of NAFLD, and is characterized by TG deposition in liver and by the presence of lipid droplets in hepatocytes (Cohen et al., 2011; Sattar et al., 2014). The formation of lipid droplets in the liver results in part from imbalances in processes that increase fatty acid (FA) content, for example, synthesis and uptake, and processes that reduce it, such as export and oxidation (Postic and Girard, 2008). Non-alcoholic steatohepatitis (NASH) is more serious than simple steatosis and patients with this condition

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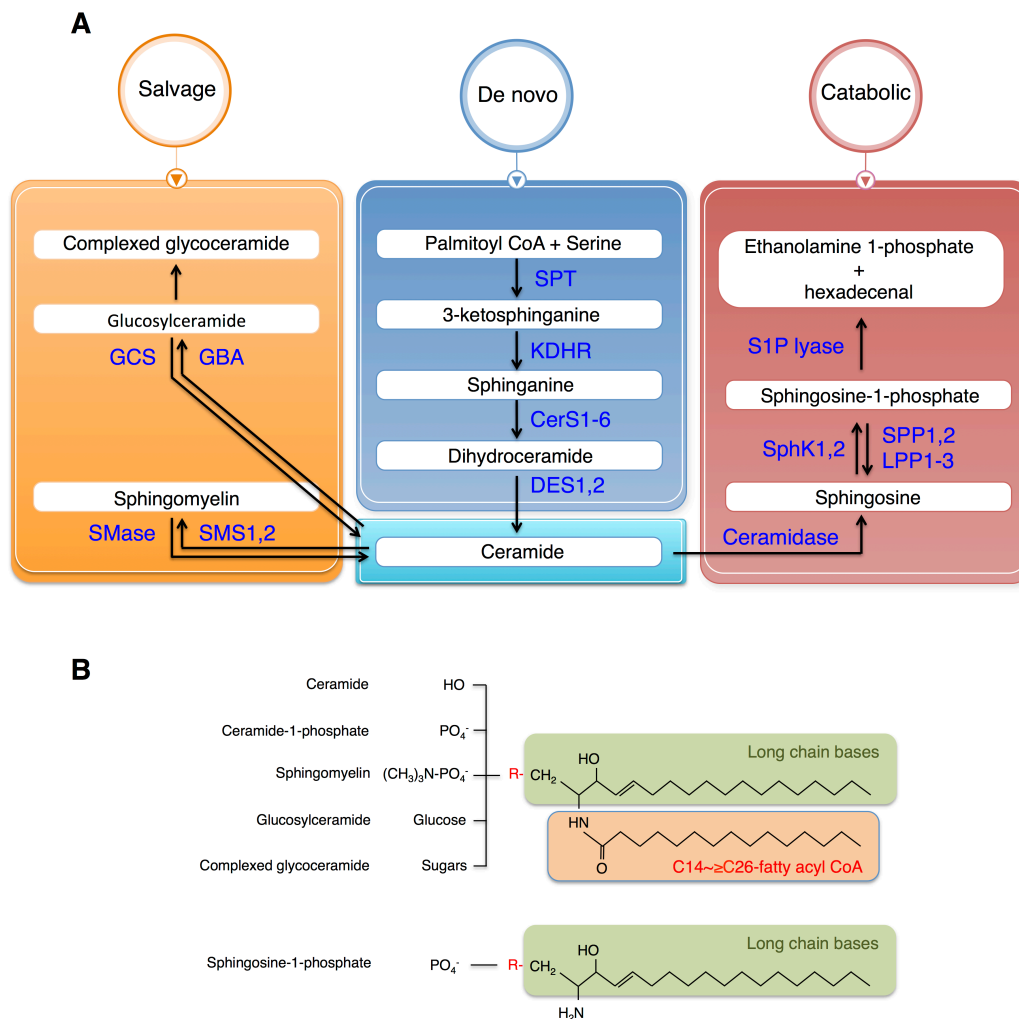
have hepatocyte injury and inflammation, which is usually accompanied by pericellular fibrosis. Adverse outcomes from NASH include cirrhosis, liver failure, and hepatocellular carcinoma (Lindenmeyer and McCullough, 2018; Rinella and Sanyal, 2016). Despite concerted efforts to elucidate the mechanism of NAFLD, it is not well understood. Likewise, despite attempts to identify therapeutic agents, no effective therapeutic agent has been approved for NASH.

When the energy needs of the body are met and no additional FFAs can be stored, excess FFAs are shunted into the nonoxidative sphingolipid synthesis pathway. Elevated sphingolipid metabolites, particularly ceramide and sphingosine

1-phosphate (S1P), are signaling molecules that indicate a cellular overnourished status. Upon reception of the signal, cells adapt to the oversupply of FA (Chaurasia et al., 2019; Rohrbach et al., 2017). In this review, we discuss the current understanding of the mechanism by which ceramide and S1P alter cellular metabolism and contribute to development of hepatosteatosis and fibrosis.

### Sphingolipid metabolism as a sensor for FA surplus

Sphingolipid metabolism is highly coordinated by a complex network of interconnected pathways, and not simply by the availability of FA substrate. The major biosynthetic site



**Fig. 1. Sphingolipid synthesis pathways and structures.** (A) Ceramide is generated by a *de novo* synthetic pathway and further metabolized via a salvage pathway. Once synthesized, ceramide is converted to either glucosylceramide or sphingomyelin by adding glucose or phosphocholine, respectively. Ceramide is degraded via a catabolic pathway to sphingosine is phosphorylated by sphingosine kinase (SphK), which can be degraded by S1P lyase. (B) Ceramide is synthesized by adding fatty acyl CoA to the long chain bases (sphingosine or sphinganine) and further metabolized to ceramide 1-phosphate (by phosphorylation), sphingomyelin (by adding phosphocholine), glucosylceramide (by adding glucose), and complexed glycosphingolipid (by adding various sugars). Ceramide is also degraded to sphingosine, which can be phosphorylated to S1P. GCS, glucosylceramide synthase; GBA, glucocerebrosidase; SMase, sphingomyelinase; SMS, sphingomyelin synthase; SPT, serine palmitoyltransferase; KDHR, 3-keto-dehydrosphingosine reductase; CerS, ceramide synthase; DES, dihydroceramide desaturase; S1P lyase, sphingosine-1-phosphate lyase; SphK, sphingosine kinase; SPP, S1P-specific phosphatases; LPP, lipid phosphate phosphatase.

for sphingolipids is the endoplasmic reticulum (ER), where FA and amino acids are condensed to form ceramides (Fig. 1) (Merrill, 2002). The condensation of ceramide is a major branching point in the pathway; it may be used in the synthesis of S1P or converted to other complex sphingolipids including sphingomyelins and gangliosides. The enzymes converting ceramides into complex sphingolipids are localized in the Golgi apparatus. *De novo* synthesis of sphingolipids is initiated from condensation of serine and palmitoyl CoA by serine palmitoyltransferase (SPT) to produce 3-ketosphinganine, followed by a series of reactions involving the enzymes 3-ketosphinganine reductase, ceramide synthase (CerS), and dihydroceramide desaturase (DES) to produce ceramide. Dihydroceramides and ceramides are transported to the Golgi apparatus and used as substrates for the enzymes that synthesize complex sphingolipids. Specifically, these include sphingomyelin by sphingomyelin synthases, gangliosides by glucosylceramide synthase, and ceramide 1-phosphate by ceramide kinase.

Another important route for ceramide metabolism is generation of S1P. In this pathway, ceramide is deacylated by ceramidases to produce sphingosine. Phosphorylation of sphingosine is catalyzed by sphingosine kinases (SphK1, 2) to generate S1P. Newly synthesized S1P is transported out of the cell by an ATP-binding cassette (ABC) transporters or by a member of the major facilitator superfamily member, spinster 2 (Spns2) (Nishi et al., 2014; Takabe and Spiegel, 2014). After export, S1P binds to one of five S1P-specific G protein coupled receptors (S1PR1-5) and activates diverse cellular responses (Maceyka and Spiegel, 2014; Pyne and Pyne, 2010). Cellular S1P levels are tightly regulated by sphingosine levels, SphKs, and the enzymes that metabolize S1P, which include S1P lyase, two S1P-specific phosphatases (SPP1-2), and three phosphate phosphatase (LPP1-3) (Maceyka et al., 2012). S1P acts as both an extracellular and intracellular signal. These have different biological functions depending on the site of generation of the SphK involved (Schwalm et al., 2013).

#### Ceramide is a regulatory messenger for excess FFA

A number of researchers have suggested that ceramide synthesis can be activated by increased FFAs (Samad et al., 2006; Schilling et al., 2013); and it has been suggested that ceramide synthesis regulates uptake of FFA. Uptake and esterification of FFAs are important in initiating the production and action of ceramide and overexpression of acid ceramidase in the liver reduced not only C16- and C18-ceramides but also CD36, the FA transporter (Xia et al., 2015). Overexpression of acid ceramidase also downregulated the activity of the atypical protein kinase C $\zeta$  (PKC $\zeta$ ), which is activated by ceramide and stimulates lipid uptake. The finding that a ceramide analogue enhanced translocation of CD36 to the membrane via a PKC $\zeta$ -dependent mechanism was interpreted as evidence that ceramide regulates FA uptake and esterification. Chaurasia et al. (2019) recently demonstrated that mice deficient in DES1 were protected from hepatic steatosis. Translocation of CD36 was stimulated by ceramide in cultured hepatocytes.

Since ceramide synthesis may reduce excess FFA, it may activate esterification of FFA into TGs. Indeed, sterol response element binding proteins (SREBPs), which are major reg-

ulators of TG and cholesterol synthesis, were activated by exogenous C16-ceramides and hepatic expression of Srebf1 and its downstream targets for FA biosynthesis including FAS and FA elongation such as Elovl6 (Jiang et al., 2015). The possible mechanism implicates atypical PKCs such as PKC $\alpha$  and  $\zeta$  which are ceramide effectors and inducers for hepatosteatosis and hypertriglyceridemia in mice (Chen et al., 2019; Taniguchi et al., 2006). In addition, the finding that ceramide analogues inhibited isoproterenol-stimulated phosphorylation of hormone-sensitive lipase (HSL) suggested that ceramide inhibits release of FFAs from TGs (Turpin et al., 2014). Collectively, these data suggest that ceramide activates TG synthesis to relieve the FFA burden and prevents FA release from lipid droplets.

Although ceramide enhanced FA entry into cells, it inhibited the uptake of glucose (Summers et al., 1998; Wang et al., 1998). The primary effect of ceramide on glucose uptake appears to be to inhibit the insulin-responsive translocation of the GLUT4 glucose transporter to the plasma membrane, by blocking insulin-mediated phosphorylation of Akt, a serine/threonine kinase involved in insulin action, anabolic signaling, and cell survival (Hajduch et al., 2001; Summers et al., 1998; Wang et al., 1998). PKC $\zeta$  activated by ceramide, phosphorylates Akt on a third inhibitory site in the enzyme's PH domain, which reduces the kinase's affinity for phosphoinositides and prevents its PI3 kinase-dependent activation (Powell et al., 2003). In addition, ceramide-activated protein phosphatase 2A (PP2A) enhances dephosphorylation of Akt (Zinda et al., 2001). The relative contribution of either PKC $\zeta$  or PP2A pathway is dependent on cell type.

Collectively, based on these findings, we suggest that ceramide regulates lipid and glucose metabolism by modulating gene expression and signaling effectors. This mechanism is the adaptation process of the substrate oxidation to adjust to lipid-overload condition.

## ROLES OF SPHINGOLIPIDS IN HEPATOSTEATOSIS AND FIBROSIS

### Ceramides in hepatosteatosis

Most TGs contain the saturated C16-FA, palmitate. Palmitate is a critical factor in lipotoxicity and a metabolic syndrome. Palmitate treatment *in vitro* not only increases inflammation, apoptosis, ER stress, insulin resistance, but also promotes synthesis of C16-ceramide (Gao et al., 2012; Schilling et al., 2013; Watt et al., 2012). Myricetin, which inhibits *de novo* sphingolipid synthesis, protected subjects from diet-induced accumulation of TG in the liver and obesity (Kurek et al., 2013; Yang et al., 2009). This protection may have been due to the reduction of total ceramide and sphingosine (Yang et al., 2009). In animal models of NAFLD, total hepatic ceramides were not changed, but the length of their acyl chain changed from C24-ceramides to C16-ceramides (Turner et al., 2013; Turpin et al., 2014). Furthermore, mice lacking CerS5 and CerS6 were resistant to a high fat diet (HFD)-induced steatosis and obesity, suggesting that C16-ceramide formation by CerS5 and CerS6 is important in the development of both fatty liver and obesity (Gosejacob et al., 2016; Turpin et al., 2014). In contrast, CerS2 heterozygote (+/-)

mice had more C16-ceramides than the wild type (WT); they also impaired FA oxidation and aggravated fatty liver after HFD (Raichur et al., 2014). PPAR- $\gamma$  and its downstream target, CD36 expression, were downregulated and FA oxidation suppressed in livers from CerS6 knock-out (KO) mice compared to livers from the WT control, but both were increased in the liver from CerS2 heterozygous KO mice. Exogenous administration of C24-ceramide reduced PPAR- $\gamma$  expression (Li et al., 2013; Park et al., 2014) and increased C16-ceramide also resulted in mitochondrial dysfunction, generation of reactive oxygen species (ROS) and caused ER stress (Kim et al., 2019; Zigdon et al., 2013). This is likely evidence that the balance between C16-ceramide and C24-ceramide is important in PPAR- $\gamma$  signaling, mitochondrial function, ROS generation, FA oxidation, and ER stress. Recently, Chaurasia et al. (2019) deleted dihydroceramide desaturase-1 (DES1), which inserts a conserved double bond into the backbone of ceramides. DES1 deficient mice showed less hepatic ceramides and serum ceramide than the WT mice, resulting in improved insulin resistance and hepatic steatosis in leptin deficiency (ob/ob) mice.

Sphingomyelin (SM) is one of the most abundant sphingolipids and it can serve as a reservoir to regulate the amount of ceramide by sphingomyelinase (SMase). There are three SMases that hydrolyze sphingomyelin to ceramide and phosphocholine. Expression of acid sphingomyelinase (aSMase) is increased in NAFLD (Longato et al., 2012; Moles et al., 2010) and aSMase deficiency protects from not only diet-induced accumulation of TGs and ER stress, but also alcohol-induced steatohepatitis (Deevska et al., 2009; Fucho et al., 2014). Furthermore, aSMase inhibition by amitriptyline prevented HFD-induced steatosis, inflammation, and fibrosis (Fucho et al., 2014). aSMase is overexpressed in the liver of NASH patients; aSMase regulates autophagy and lysosomal membrane permeabilization (Fucho et al., 2014). Although neutral SMase2 (nSMase2) is the main nSMase in the SMase family and it can be activated by inflammatory cytokines or glutathione (GSH) depletion (Rutkute et al., 2007a; 2007b), there are few studies of this in NAFLD. nSMase2 deficiency mice have a dwarf phenotype, with joint deformation, shortened long bones, and lower levels of growth hormone than the WT, so it seems clear that nSMase2 is important in bone formation (Stoffel et al., 2007). The nSMase2 inhibitor, GW4869, reduced inflammation and atherosclerosis formation in ApoE-/- mice and increased insulin resistance in C2C12 cells (Lallemand et al., 2018; Verma et al., 2014). Recently, GW4869 is emerging as important exosome secretion regulator, which can modulate inflammation through the NF- $\kappa$ B pathway and insulin sensitivity (Gao et al., 2016; Ying et al., 2017). Thus, nSMase2 may have an indirect role in the development of NAFLD.

Most SM is generated by sphingomyelin synthase 1 (SMS1). Therefore, it is not surprising that SMS1-null mice exhibited moderate neonatal lethality and severe pancreatic dysfunction (Yano et al., 2011). This precludes the use of these mice in obesity experiments. Instead, mice deficient in sphingomyelin synthase (SMS 2), which are more sensitive to insulin and diet-induced obesity than WT mice (Mitsutake et al., 2011), have less PPAR- $\gamma$  and its downstream target CD36,

and have smaller lipid droplets. Overexpression of SMS2 in the liver had the opposite effect: it stimulated FA uptake, resulting hepatic steatosis (Li et al., 2013). Although there is a consensus that ceramide levels correlate positively with insulin resistance and development of fatty liver, SMS2 liver-specific transgenic mice had less ceramide and were more susceptible to diet-induced fatty liver formation than WT mice, while mice deficient in SMS2 had more ceramide than the WT mice and were more susceptible to diet-induced fatty liver formation. Since SMS2 is located in plasma membrane and a novel regulator of a plasma membrane microdomain, SMS2 appears to regulate FA uptake via Caveolin1 and CD36, which are also located in the microdomain (Mitsutake et al., 2011). Inhibition of glucosylceramide synthase (GCS), another enzyme that converts ceramide to glucosylceramide, also protected against HFD-induced fatty liver. These mice also had less SREBP-1c and its downstream targets, FA synthase (FAS), and stearoyl-CoA desaturase-1 (SCD-1) than mice in which GCS had not been inhibited, but PPAR- $\alpha$  and PPAR- $\gamma$  were unaffected (Zhao et al., 2009). The exact mechanism of how GCS inhibition affects the development of fatty liver and insulin resistance is unclear, but the reduced ganglioside GM3, which plays an important role in insulin resistance (Tagami et al., 2002; Yamashita et al., 2003), could increase insulin sensitivity.

### Sphingosine 1-phosphate in steatosis and obesity

Accumulating evidence is consistent with the proposal that S1P levels correlate with the insulin resistance and occurrence of hepatic steatosis. Recently, evidence suggested to workers that SphKs and S1P are major players in lipid accumulation in liver (steatosis) and lipid storage in adipose tissue (obesity) (Sanyal and Pacana, 2015). A lipidomic study using a model for NAFLD in which obesity was induced in mice with a HFD provided evidence that increasing sphingolipid resulted in sphingosine and S1P levels in the liver that were comparable to those in mice supplied with a normal chow diet. It may be that the HFD-induced *de novo* sphingolipid synthesis is associated with NAFLD progression (Sanyal and Pacana, 2015).

The role of sphingosine kinase 1 (SphK1) in NAFLD is controversial. SphK1 expression increased in hepatic steatosis and SphK1-deficient mice are protected against hepatic steatosis. S1P generated from SphK1 action regulates PPAR- $\gamma$  through the S1PR-Akt-mTOR signaling pathway (Chen et al., 2016a). However, SphK1-null mice had increased insulin resistance, attributed to reduced insulin production resulting from increased lipotoxicity-induced  $\beta$ -cell death (Qi et al., 2013). Moreover, SphK1 is involved in inflammatory cytokine production in a NF- $\kappa$ B pathway-dependent manner (Geng et al., 2015). SphK1 overexpression induced with the adenovirus AdSphK1 resulted in lower basal glucose level and improved glucose tolerance as well as fatty liver in diabetic mice compared to mice with normal expression of SphK1 (Ma et al., 2007). In contrast, though, in another study mRNA of SphK1 and SphK activity decreased in HFD-fed mice liver compared to mice fed normal diets (Kowalski et al., 2015) and SphK1 overexpression by adeno-associated virus (AAV)-SphK1 had reduced hepatic triglyceride in mice fed low-fat diets, but did not affect steatosis or insulin resistance in mice fed HFD

(Kowalski et al., 2015). Although SphK2 expression does not increase during the development of fatty liver (Chen et al., 2016a), overexpression of SphK2 achieved with an adenovirus increased S1P, but reduced sphinganine; sphingosine; C16- and C18-ceramides; and C16- and C18-sphingomyelins. Hepatic triglyceride and cholesterol levels were reduced and FA oxidation was activated upon SphK2 overexpression (Lee et al., 2015) compared to mice normally expressing SphK2. Additional evidence provide support for this notion. SphK2 KO mice fed a HFD developed hepatosteatosis, while those fed a normal diet did not (Nagahashi et al., 2015). Therefore,

it may be that elevated S1P in the nucleus, via the action of SphK2, is linked to reduced fat accumulation in liver, and that SphK2 is a key regulator of FA oxidation and TG metabolism.

The fingolimod FTY720 is a therapeutic drug for multiple sclerosis and the autoimmune disease. The drug acts as a functional antagonist of S1PR1 to induce S1PR1 degradation (Brinkmann et al., 2010). In mice fed a high-calorie diet to induce NASH, FTY720 administration reduced body and liver weight, and these effects were accompanied by decreasing hepatocyte ballooning, hepatic inflammation, and fibrosis in liver compared to mice fed a normal diet (Mauer

**Table 1.** The effects of sphingolipids changes on fatty liver and insulin resistance

Pathway	Chemical treated or KO mice	Insulin resistance	Fatty liver	Phenotype	Reference
De novo ceramide biosynthesis	Myriocin	Improved	Improved	Weight gain after HFD ↓ Serum ceramide ↓ Insulin signaling in liver and muscle ↑ Energy expenditure ↑ (UCP3 ↑, SOCS-3 ↓ in adipose tissue)	(Holland et al., 2007; Kurek et al., 2013; Ussher et al., 2010; Yang et al., 2009)
	DES1 KO mice	Improved	Improved	Weight gain in ob/ob mice ↓ Hepatic C16, C18, C20, C22, C24-Cer ↓ Serum C16, C18, C20, C22, C24-Cer ↓ White adipose tissue mass ↓	(Chaurasia et al., 2019)
	CerS6 KO mice	Improved	Improved	Weight gain after HFD ↓ Hepatic p-Akt, p-GSK3 ↑ Energy expenditure ↑ β-oxidation capacity in brown adipose tissue ↑ Hepatic β-oxidation ↑ PPAR-γ, CD36 ↓	(Turpin et al., 2014)
	CerS5 KO mice	Improved	Improved	Weight gain after HFD ↓	(Gosejacob et al., 2016)
	CerS2 heterozygote (+/-) mice	Aggravated	Aggravated	Serum cholesterol ↑ Impaired lipid oxidation Impaired electron transport chain activity	(Raichur et al., 2014)
	Salvage pathway	nSMase2 KO mice	No study	No study	Dwarfism phenotype Joint deformation Short statured long bones Growth hormone ↓
aSMase KO mice		Improved	Improved	Hepatic stellate cells proliferation ↓ Liver fibrosis after bile duct ligation ↓ ER stress ↓	(Fucho et al., 2014)
aSMase inhibition (amitrypsine, imipramine, desipramine)		Improved	Improved	Inflammation ↓ Serum ceramide ↓ C16-Cer, C24-Cer ↓ p-Akt, p-p70S6K ↑ p-p38, p-JNK ↓	(Fucho et al., 2014; Jin et al., 2013; Liangpunsakul et al., 2012)
GCS inhibition (Genz-123346, Genz-112638, AMP-DNM)		Improved	Improved	FAS, SCD-1, ACC1 ↓ p-Insulin receptor-β, p-mTOR ↑ GM3 ↓	(Aerts et al., 2007; Zhao et al., 2007; 2009)
Sphingomyelin pathway	SMS1 KO mice	No study	No study	Mitochondrial dysfunction Impaired insulin secretion	(Yano et al., 2011)
	SMS2 KO mice	Improved	Improved	Weight gain after HFD ↓ PPAR-γ, CD36 ↓	(Mitsutake et al., 2011)
	SMS2 transgenic mice	No study	Aggravated	CD36 ↑	(Li et al., 2013)

**Table 1.** Continued

Pathway	Chemical treated or KO mice	Insulin resistance	Fatty liver	Phenotype	Reference
Catabolic pathway	SphK1 null mice	Aggravated	Improved	Hepatic triglyceride, cholesterol ↓ Hepatic S1P ↓ PPAR-γ, CD36, UCP2, Cidea ↓ Pancreatic β-cell mass in HFD-fed ↓ Insulin production in HFD-fed ↓	(Chen et al., 2016a; Qi et al., 2013)
	SphK1 overexpression by AdSphK1	Improved	Improved	Hepatic triglyceride ↓ p-Akt, p-GSK3 ↑	(Ma et al., 2007)
	SphK2 overexpression by AdSphK2	Improved	Improved	p-Akt ↑ C16, C18, C24:1-Cer ↓ Sphingosine, sphinganine ↓ PPAR-α, CPT1, ACOX1 ↑	(Lee et al., 2015)
	FTY720 (S1PR1 antagonist)	Improved	Improved	Macrophage infiltration ↓ Ly6-C (monocyte-derived macrophages marker), CCR2 (C-C chemokine receptor type 2) ↓ α-smooth muscle actin ↓ C16-, C24:1-ceramide ↓ Fatty acid synthase ↓ S1P, dihydro-S1P ↓	(Mauer et al., 2017; Rohrbach et al., 2019)
	S1PR2 deficiency mice	No study	Aggravated	H3K9 acetylation ↓ H4K5 acetylation ↓ H2BK12 acetylation ↓	(Nagahashi et al., 2015)

et al., 2017). When mice fed a western diet supplemented with sweet water, the administration of FTY720 alleviated hepatosteatosis, and this was accompanied by decreasing hepatic inflammation and sphingolipid species—specifically, ceramide, dihydroceramide, S1P, and dihydro-S1P (Rohrbach et al., 2019). In contrast to mice deficient in S1PR1, the mice lacking S1PR2 rapidly developed fatty livers on a HFD (Nagahashi et al., 2015). From this evidence, it may be that the S1P receptor isoforms contribute differently to the development of fatty livers. The effects on fatty liver and insulin resistance caused by manipulation of sphingolipid levels are summarized in Table 1.

#### Implication of ceramides and S1P in liver fibrosis

Liver fibrosis is a chronic liver disease that results from excess production of extracellular matrix proteins (Bissell, 1998), as a result of multiple injuries, functional wound healing, or chronic liver disease. It can lead to cirrhosis or liver cancer (Kisseleva et al., 2012; Kitatani et al., 2015). During fibrosis, hepatocytes undergo apoptosis and activate hepatic stellate cells by activating Kupffer cells, which release proinflammatory cytokines (Higuchi and Gores, 2003; Pessayre et al., 2002). Activated hepatic stellate cells secrete extracellular matrix to fill the space called the Disse space, and they proliferate and replace dead hepatocytes with fibrous scar tissue; this is fibrosis (Bataller and Brenner, 2005; Schuppan and Afdhal, 2008; Shea and Tager, 2012). Thus, drugs that block or inhibit hepatic stellate cell activation may be effective in preventing fibrosis (Rippe and Brenner, 2004).

#### Ceramide and liver fibrosis

As ceramide accumulates, cells undergo apoptosis and trigger the fibrotic events. Fibrosis is a defense mechanism to protect the tissue from lysing cells. In mice on which fibrosis was induced with carbon tetrachloride treatment, the total amount of ceramide was increased in both plasma and liver (Ichi et al., 2007), and mice lacking acid SMase had lower ceramide levels than mice with this enzyme (Mari et al., 2008). Acid sphingomyelinase activates hepatic stellate cells, which promote fibrogenesis through promoting migration of the cells and extracellular matrix secretion (Moles et al., 2010). Ceramide regulates the expression of collagen genes, important components of the extracellular matrix, via a mechanism of “regulated intra membrane proteolysis”. Cyclic AMP response element 3 like 1 (CREB3L1) is a transcriptional factor, which regulate collagen synthesis. CREB3L1 is cleaved by two Golgi-localized protease, site-1 and site-2 proteases (S1P/S2P), leading to enter the nucleus where it binds Smad4 and then upregulates transcription of genes for assembly of collagen-containing extracellular matrix (Chen et al., 2016b). Ceramides alter the orientation of TM4SF20, a protein blocking the access of S1P/S2P to CREB3L1, and activate fibrogenic processes (Chen et al., 2016b; Denard et al., 2012). In a recent study, administration of myriocin significantly reduced ceramide levels and reduced liver inflammation and fibrosis (Jiang et al., 2019). Thus, ceramide is believed to be important in regulating apoptosis of hepatocytes. It is also a potential, major target for the treatment of NASH and fibrosis.

#### S1P and liver fibrosis

S1P regulates the expression of various extracellular matrices

during liver fibrosis (Li et al., 2009a). S1P also activates the proliferation and migration of hepatic stellate cells *in vitro* and increases the expression of extracellular matrix proteins, such as  $\alpha$ -smooth muscle actin and collagen I and III (Al Fadel et al., 2016; Friedman, 2008; Gonzalez-Fernandez et al., 2017). In liver fibrosis studies, the levels of S1P were elevated consequent to increased hepatic SphK1 expression compared to the control tissue; this was observed both in fibrous liver tissue from mouse and human patients (King et al., 2017; Li et al., 2011). The expression level of Spns2 mRNA, which encodes a transporter of S1P, was elevated in fibrotic human liver compared to normal liver, indicating increased export of S1P and its binding to specific receptors, leading to fibrosis and inflammation (Sato et al., 2016). In a recent report, workers analyzed 95 patients with end-stage liver disease and observed that patients with low concentrations of plasma S1P had a poor prognosis (Becker et al., 2017). It seems, then, that S1P has a complex role in the development of advanced liver fibrosis and cirrhosis.

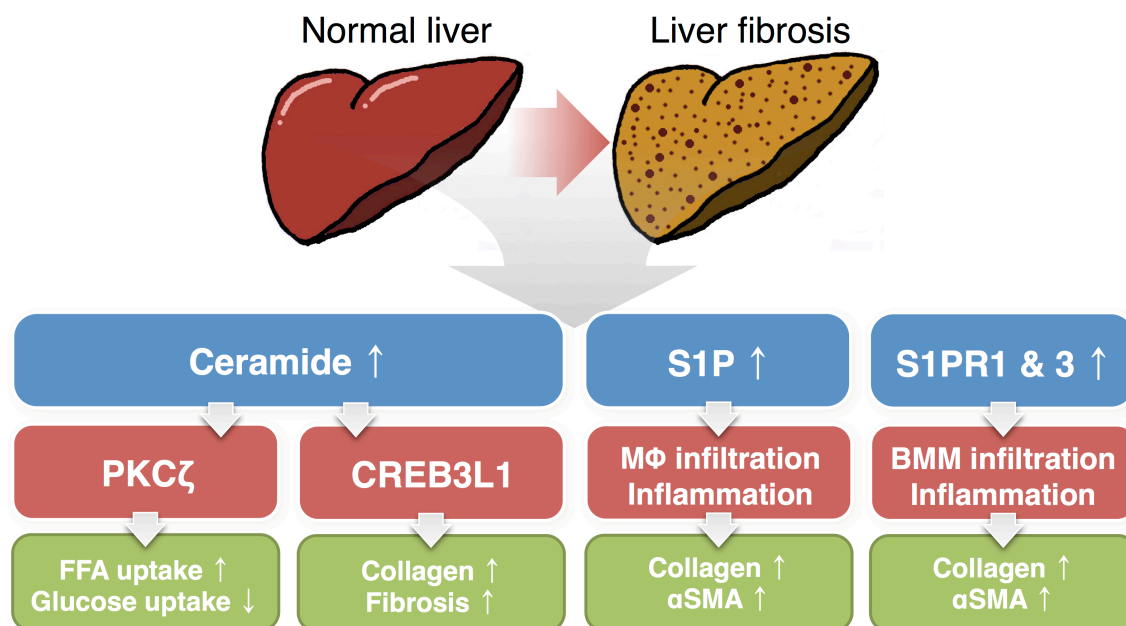
#### S1PRs and liver fibrosis

Two S1P receptors, S1PR1 and S1PR3, are considered to be the two major S1PRs that are important in liver fibrosis. Their levels were also elevated in cholestasis-induced liver fibrosis and in human fibrotic samples, whereas the S1PR2 levels were decreased, compared to the appropriate normal controls (Li et al., 2011; Xiu et al., 2015). Antagonists of S1PR1

and S1PR3 blocked upregulation of *Ang1* and alleviated fibrosis in the damaged liver, whereas the S1PR2 antagonist had no effect in angiogenesis (Yang et al., 2013). Silencing of S1PR3 diminished not only the ability of bone marrow-derived cells to migrate to the liver but also their transdifferentiation into myofibroblast-like cells (Li et al., 2009a). Recently, human embryonic lethal abnormal visual protein (HuR) was induced during liver fibrosis via S1P and increased expression of S1PR3. HuR, an mRNA binding protein, affects the vitality of bone marrow-derived cells and further stabilize their mRNA (Chang et al., 2017). On the other hand, a KO mutant of S1PR2 in animal models of liver fibrosis protects mice from the development of fibrosis (Ikeda et al., 2009), and the expression of S1PR2 was reduced in the liver analysis from the patients with liver fibrosis (Li et al., 2011). We suggest that further studies are needed to identify the roles of S1P and S1PRs in the fibrosis process induced by various cells. Major sphingolipids and the mechanism for development of fatty liver and fibrosis are summarized in Fig. 2.

#### Therapeutic targets in the treatment and prevention of liver fibrosis

Tracking and altering the signaling pathway of S1P may aid in the treatment of liver fibrosis. For example, the discovery of fingolimod, also known as FTY720 and a modulator of S1PR1, is promising. This drug has been approved by the U.S. Food and Drug Administration (FDA) and is the first oral drug



**Fig. 2. The roles of ceramide and S1P in liver fibrosis.** During liver fibrosis, ceramide and S1P levels are elevated. Ceramide promotes PKC $\zeta$  activation, which induces CD36-mediated fatty acid uptake (Xia et al., 2015) and disturbs glucose uptake (Powell et al., 2003). Ceramide also stimulates CREB3L1 cleavage, which activates fibrogenic processes (Chen et al., 2016b; Denard et al., 2012). S1P induces Kupffer cell infiltration, which increases expressions of collagen and  $\alpha$ -smooth muscle actin (Al Fadel et al., 2016; Friedman, 2008; Gonzalez-Fernandez et al., 2017). S1PR1 and S1PR3 is also involved in bone marrow-derived macrophage/monocytes migration to the liver (Li et al., 2011; Xiu et al., 2015). PKC $\zeta$ , protein kinase C zeta; FFA, free fatty acids; CREB3L1, cAMP responsive element binding protein3 like 1; S1P, sphingosine 1-phosphate; M $\phi$ , macrophage;  $\alpha$ SMA,  $\alpha$ -smooth muscle actin; S1PR, S1P receptor; BMM, bone marrow-derived macrophage/monocytes.

**Table 2.** The candidate drugs/agents of antifibrotic activity

Action	Drug/agent	Reference
Sphingosine kinase inhibitor	PF543 (SphK inhibitor)	(Gonzalez-Fernandez et al., 2017)
	SKI-II (SphK inhibitor, non-selective)	(Yang et al., 2013)
	N,N-dimethylsphingosine (DMS, SphK inhibitor)	(Brunati et al., 2008; Wang et al., 2017b; Xiu et al., 2015)
S1P receptor agonist/antagonist	FTY720 (S1PR1 and S1PR3 agonist)	(Brunati et al., 2008; King et al., 2017; Kong et al., 2014)
	VPC23019 (S1PR1 and S1PR3 antagonist)	(Brunati et al., 2008; Yang et al., 2012; 2013)
	SEW2871 (S1PR1 agonist)	(Ding et al., 2016)
	W146 (S1PR1 antagonist)	(King et al., 2017; Liu et al., 2011; Yang et al., 2012; 2013)
	JTE-013 (S1PR2 antagonist)	(Kageyama et al., 2012; Wang et al., 2017a; Xu et al., 2016; Yang et al., 2015)
	Suramin (S1PR3 antagonist)	(Li et al., 2009a; 2009b)
	KRP203 (FTY720 analog)	(Kaneko et al., 2006; Khattar et al., 2013)
	CAY-10444 (S1PR3 antagonist)	(Yang et al., 2015)
Other inhibitor	VPC24191 (S1PR1 and S1PR3 antagonist)	(Al Fadel et al., 2016)
	Pertussis toxin (PTX; G-protein-coupled receptor signaling inhibitor)	(Brunati et al., 2008; Gonzalez-Fernandez et al., 2017; Yang et al., 2015)
	Melatonin (melatonin receptors agonist)	

that effectively treats recurrent multiple sclerosis. The development of inhibitors of S1P signaling and approaches that target enzymes in the sphingolipid pathway are novel fields in the search for efficient antifibrotic drugs (Dyckman, 2017; Park and Im, 2017). The major candidate drugs that mediate antifibrotic activity through the regulation of S1P or its receptors reported so far are summarized in Table 2.

## CONCLUSION

Ceramides are the nutrient sensors that alleviate the FFA oversupply, inhibit glucose utilization, and activate deposition of TGs in the liver. Acting independently of ceramides, S1P activates cellular signaling via S1PR binding and has important roles in hepatic pathologies. While pharmacological intervention of sphingolipid biosynthesis for NAFLD is promising, more detailed understanding of the pathways is needed. It is expected that the second generation of therapeutics for liver diseases can be pursued in light of these metabolic pathways.

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## AUTHOR CONTRIBUTIONS

W.J.P. and T.S.P. contributed to the conception and design of the manuscript. J.H.S., G.T.K., W.J.P., and T.S.P. wrote the manuscript. J.H.S. and G.T.K. summarized tables and W.J.P. designed figures. W.J.P. and T.S.P. reviewed and edited the final manuscript.

## CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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