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Review

# Ceramides – Lipotoxic Inducers of Metabolic Disorders

Bhagirath Chaurasia[1] and Scott A. Summers[1,*]

**In obesity and dyslipidemia, the oversupply of fat to tissues not suited for lipid storage induces cellular dysfunction that underlies diabetes and cardiovascular disease (i.e., lipotoxicity). Of the myriad lipids that accrue under these conditions, sphingolipids such as ceramide or its metabolites are amongst the most deleterious because they disrupt insulin sensitivity, pancreatic β cell function, vascular reactivity, and mitochondrial metabolism. Remarkably, inhibiting ceramide biosynthesis or catalyzing ceramide degradation in rodents ameliorates many metabolic disorders including diabetes, cardiomyopathy, insulin resistance, atherosclerosis, and steatohepatitis. Herein we discuss and critically assess studies that identify sphingolipids as major contributors to the tissue dysfunction underlying metabolic pathologies, highlighting the need to further decipher the full array of benefits elicited by ceramide depletion.**

## Introduction

The term lipotoxicity was coined in the early 1990s by Roger Unger, who postulated that excessive delivery of lipid was the initial insult in diabetes and metabolic disorders [1,2]. The idea was predicated on the observation that the metabolic defects present in individuals susceptible to diabetes, such as peripheral insulin resistance, hyperinsulinemia, and impaired glucose-stimulated insulin secretion, could all be recapitulated by increasing the delivery of fatty acids to skeletal muscle and pancreatic islets. Data generated in the subsequent 20 years strongly support the Unger lipotoxicity model. Experimental manipulations that limit the storage capacity of adipose tissue and/or promote ectopic lipid deposition invariably give rise to metabolic disorders. Moreover, therapies that ameliorate metabolic diseases promote safe storage (e.g., thiazolidinediones) or oxidation (e.g., metformin) of fat [3].

Lipids entering the cell are either metabolized in mitochondria or converted into complex lipids through various biosynthetic pathways resident in the endoplasmic reticulum (ER). The major Kennedy pathway produces di- and triacylglycerols and glycerophospholipids. Although triglycerides are clearly a good marker of the lipotoxic condition, they are probably not harmful, and potentially are even protective. The intermediate diacylglycerol (DAG) has received considerable attention as a putative lipotoxic agent, particularly as an inducer of insulin resistance [4].

By comparison, only a fraction of lipids enter the biosynthesis pathway leading to the production of ceramide, the precursor of complex sphingolipids. In an early interventional study using pharmacological inhibitors to block ceramide biosynthesis, the Unger laboratory found that the lipotoxic effects of fatty acids on pancreatic β cells were negated by the inclusion of inhibitors of ceramide biosynthesis [5]. One such inhibitor, the serine palmitoyltransferase inhibitor cycloserine, protected Zucker diabetic fatty (ZDF) rats from the development of diabetes [5]. This

### Trends

Inhibition of ceramide biosynthesis ameliorates virtually all metabolic disorders in rodents.

Lipidomic profiling studies have generally shown increased ceramides in relation to various disease endpoints, but discordance between studies has created controversy.

Mechanisms of ceramide action include inhibition of insulin and growth factor signaling and action, impairment of mitochondrial lipid oxidation, ER stress, and induction of apoptosis.

Adiponectin elicits its broad spectrum of metabolic actions by catalyzing ceramide deacylation via ceramidase activation.

Inflammatory agents selectively upregulate the sphingolipid pathway, and this is essential for their induction of insulin resistance.

[1]Baker IDI Heart and Diabetes Institute, Melbourne, Australia

*Correspondence: scott.summers@bakeridi.edu.au (S.A. Summers).

prompted the Unger group to later describe ceramides as 'the most important of the deleterious routes' underlying lipotoxic events [6].

In the 20 years since the lipotoxicity concept was born, new tools have allowed rigorous studies of ceramides and ceramide-metabolites in metabolic disorders. A major advance has been the advent of lipidomic profiling methods by mass spectroscopy, allowing a comprehensive assessment of sphingolipids and glycerolipids that accumulate in obesity and dyslipidemia. The second, and more significant, has been the cloning of the genes required for ceramide biosynthesis and metabolism, allowing researchers to alter the profiles of endogenous sphingolipids through genetic manipulations (e.g., small interfering RNA or genetic deletions). Experiments with these new tools strongly support the initial Unger observation about the importance of ceramides in metabolic disorders. Astonishingly, implementation of pharmacological and genetic engineering approaches to reduce ceramide levels in rodents prevents the onset of insulin resistance, diabetes, steatohepatitis, hypertension, cardiomyopathy, and atherosclerosis [7–19].

## Ceramide Synthesis and Degradation

Ceramides are precursors for the predominant sphingolipids in the cell, including sphingomyelin and gangliosides. This sphingolipid family includes over 4000 distinct species (www.lipidmaps.org) that are integral components of cell membranes. Many play regulatory roles in cellular growth and function, with ceramides having received the greatest attention as initiators of a coordinated stress response (e.g., growth inhibition, inhibited anabolism, and ultimately apoptosis) [20].

Sphingolipids are not absorbed appreciably from dietary sources, but are instead produced from breakdown products of saturated fats (e.g., palmitate) and proteins (e.g., serine). These substrates enter a ubiquitous biosynthetic pathway that includes four evolutionarily conserved reactions (for details see Box 1 and Figure I in Box 1) [21]. Once generated in the ER, ceramide and dihydroceramide traffic to the Golgi where they can be converted into complex sphingolipids (e.g., sphingomyelins/dihydrosphingomyelins, glucosyl- and galactosyl-ceramides/dihydroceramides, etc). This occurs through the addition of different headgroups, such as phosphocholine to generate sphingomyelins, or sugars to make glucosyceramides, cerebrosides, and gangliosides. An interesting ceramide transfer proteins (CERT1) selectively translocates ceramides to Golgi domains for conversion into glucosylceramides [22].

Salvage pathways allow for the re-formation of ceramide from complex sphingolipids. In plasma membranes, neutral sphingomyelinases (SMases) hydrolyze the phosphocholine from sphingomyelins, converting them back into ceramides and free choline (Figure 1) [23]. In lysosomes, acidic forms of SMase and β-glucosidase 1 convert sphingomyelin and glucosylceramides, respectively, into ceramides, which can be further deacylated by ceramidases to produce sphingosine capable of re-entering the *de novo* synthesis pathway [24].

Experimental manipulations of the enzymes in this pathway in rodent models of disease have been revealing. The beneficial impact of redundant pharmacological or genetic approaches to slow rates of ceramide synthesis on a vast number of metabolic disease endpoints reveals unanticipated roles for distinct ceramides and ceramide metabolites in metabolic regulation.

## Sphingolipid Profiling in Obesity

A large number of groups have profiled sphingolipids in plasma or tissues of various disease populations, but the results have been confusing. Several groups have reported that ceramides and other sphingolipids accumulate in muscle or serum of insulin resistant patients and non-human primates [25–31]. The most detailed of these were by the Goodpaster laboratory, who observed a prominent increase in numerous ceramide species in insulin-resistant individuals.

## Box 1. Enzymes Required for Ceramide Biosynthesis

*Serine Palmitoyltransferase*

Ceramide biosynthesis begins in the endoplasmic reticulum with the condensation of palmitoyl-CoA and serine, catalyzed by the multimeric enzyme serine palmitoyltransferase (SPT), to produce 3-ketosphinganine (Figure I). SPT is composed of two essential subunits (SPTLC1 and 2), which are essential for enzyme function. On occasions this enzyme can use alternative amino acids (i.e., alanine and glycine) to produce deoxysphingolipids, which are deleterious in neurons. Mutations which alter the substrate selectivity of SPT lead to aberrant accumulation of these deoxysphingolipids and the subsequent development of a rare hereditary sensory neuropathy [113]. The inclusion of a third SPTLC subunit broadens the specificity for acyl-CoAs (e.g., myristoyl-CoA), allowing the generation of different chain-length scaffolds. These less abundant myristate-derived sphingolipids have been implicated in the dysregulation of cardiomyocyte function [114]. Modulation of SPT may also be mediated by ORMDL (ORMDL sphingolipid biosynthesis regulator) proteins initially identified in yeast and that are conserved in rodents and humans [115].

*3-Ketosphinganine Reductase*

The 3-ketosphinganine produced by the SPT reaction is a short-lived intermediate that is rapidly converted to sphinganine by 3-ketosphinganine reductase (3KSN). Less is known about the regulation of this intermediary event than the other three reactions.

*Ceramide Synthases*

The subsequent *N*-acylation of sphinganine by a family of ceramide synthases (CERS1–6) produces dihydroceramides, and much of the diversity in the sphingolipid pool results from this reaction. The six different mammalian CERS enzymes produce dihydroceramides of variable acyl-chain lengths, ranging from 14 to 34 carbon atoms [116]. These enzymes differ in substrate specificity and tissue distribution, allowing the formation of distinct sphingolipid pools in different tissues and cell types.

*Dihydroceramide Desaturases*

Dihydroceramide desaturases (DES1 and 2) insert a double-bond that imparts many of the unique biophysical properties of the sphingolipid [117]. DES1 is the dominant enzyme in most tissues, with DES2 making phytosphingolipids in the skin and gut. While dihydroceramides were once considered inert, recent studies reveal distinct and non-overlapping biological roles with the more prevalent ceramides [120].

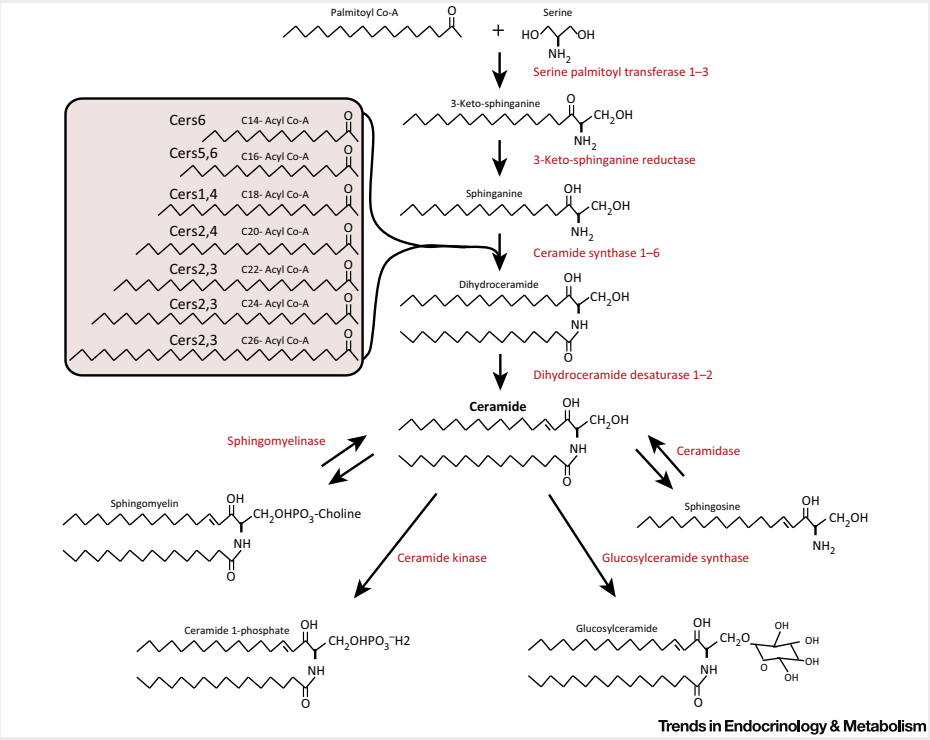


Figure I. Biochemistry of Sphingolipid Biosynthesis. Schematic illustration of three independent pathways produce ceramides: *de novo* synthesis, sphingomyelin hydrolysis, and salvage pathways.

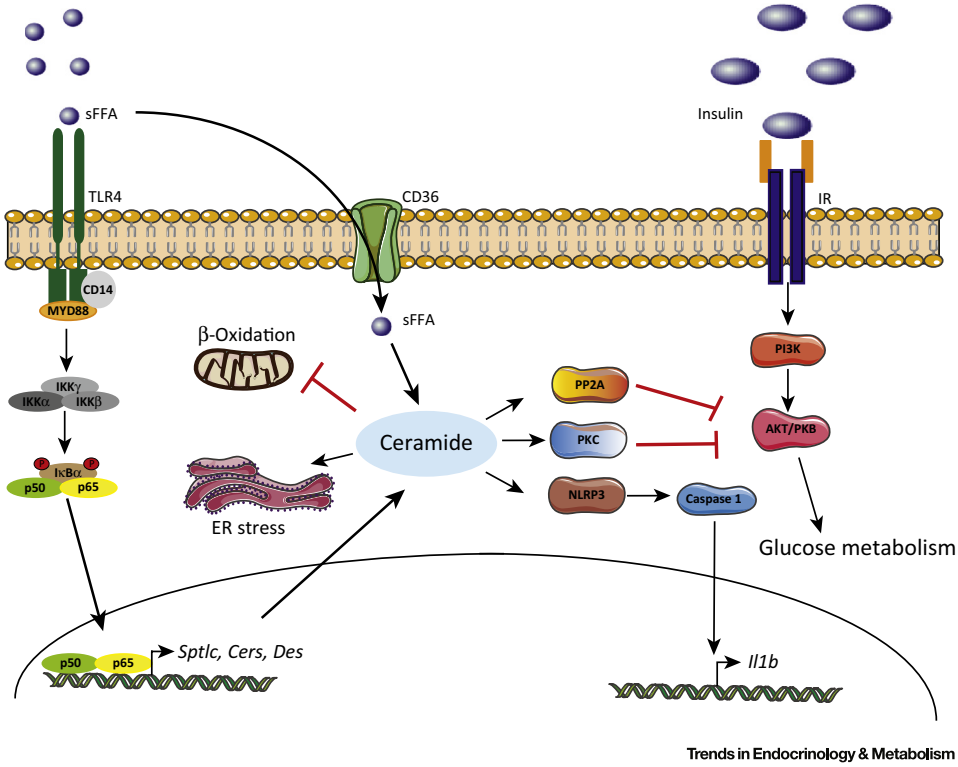


Figure 1. Ceramides and Metabolic Dysfunction. Ceramide elicits metabolic dysfunction by several mechanisms. It inhibits AKT/PKB via the intermediaries PP2A and PKCζ. Ceramides also induce ER stress, inhibit mitochondrial β-oxidation, and activate the NLRP3 inflammasome. Abbreviations: AKT/PKB, protein kinase B; CD14, cluster of differentiation 14; CD36, cluster of differentiation 36; CERS, ceramide synthase; DES1, dihydroceramide desaturase 1; ER, endoplasmic reticulum; IkBα, inhibitor of NF-κB; IKK, IkB kinase; IL-1β, interleukin 1β; IR, insulin receptor; MYD88, myeloid differentiation primary response protein 88; NLRP3, NLR family; pyrin domain containing 3; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PP2A, protein phosphatase 2A; sFFA, saturated fatty acids; SPTLC, serine palmitoyl transferase; TLR4, Toll-like receptor 4.

The changes were independent of obesity, physical fitness, free fatty acids, or DAGs [26–28]. Other groups also detected strong correlations between plasma ceramides and insulin resistance, particularly when considered in concert with levels of inflammatory cytokines [29,30]. Consensus has not been reached, however, because others have reported no change in ceramides in individuals susceptible to metabolic disorders [32,33].

A challenge in interpreting these profiling studies is that ceramides are biosynthetic intermediates that do not exist at a steady-state concentration. Little is known about how they fluctuate in response to feeding or other environmental factors, and flux determinations are not typically obtained. Moreover, the subcellular location of the crucial pool of sphingolipids that regulate cell function remains unresolved. With these temporal/spatial aspects of the sphingolipidome incompletely understood, interpretation of these findings is difficult.

## Regulation of Ceramide Synthesis and Degradation

The oversupply of palmitate and serine in states of overnutrition likely contributes to the upregulation of ceramides in obesity, but other factors also control ceramide production [34]. Hormonal cues and the microbiome exert profound metabolic effects by modifying rates of ceramide synthesis and degradation [11,12]. Fatty acids themselves alter the expression of genes involved in sphingolipid biosynthesis and metabolism [10,35,36].

## Inflammation and Ceramide Synthesis

Obesity is associated with chronic low-level inflammation. Saturated fatty acids activate or augment signaling through receptors involved in innate immunity (e.g., Toll-like receptors. TLRs), upregulating cytokine synthesis and secretion [37]. Moreover, macrophages infiltrate the expanded adipose depot, which then expresses different cytokine and other macrophage markers [38]. The inflamed environment likely works in concert with excessive nutrient availability to drive sphingolipid production.

Unbiased lipidomic screens have long-shown that inflammatory agents [e.g., TLR4 agonists, tumor necrosis factor ∝ (TNF-∝), interleukins, etc.] increase levels of sphingolipids without affecting glycerolipids such as DAG [29]. The aforementioned correlational studies show particularly tight relationships between plasma ceramides and insulin resistance in concert with levels of circulating inflammatory cytokines [29,39]. Cell-based assays reveal that blocking ceramide production can negate many other actions of inflammatory stimuli.

In particular contexts, the inflammatory environment is crucial for the upregulation of ceramides. For example, saturated fatty acids that fuel the biosynthetic pathway also activate (or amplify) signaling through TLRs, which have also been shown to be essential for lipid-induced insulin resistance [37,40,41]. In at least a subset of tissues, the presence of this TLR4 signaling network was a prerequisite for palmitate-mediated induction of ceramides (Figure 2) [11,42,43].

A mechanism by which ceramides might contribute to inflammation-induced insulin resistance and diabetes is through the activation of the nucleotide-binding domain, leucine-rich-containing family, pyrin domain containing 3 (NLRP3) inflammasome [44–46]. This putative ‘ceramide

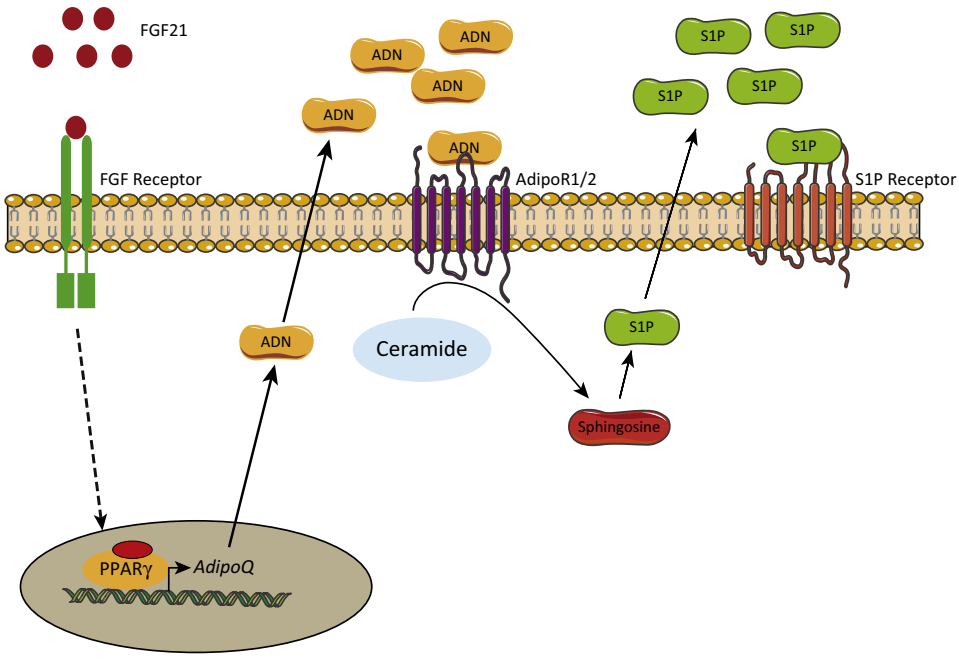


Figure 2. Regulation of Ceramide Degradation by FGF21 and Adiponectin. The insulin-sensitizing and metabolically protective agents FGF21 and adiponectin elicit at least some of their functions through the activation of a ceramidase to generate sphingosine and S1P. Abbreviations: *AdipoQ*, adiponectin gene; AdipoR1/2, adiponectin receptor; ADN, adiponectin protein; FGF21, fibroblast growth factor 21; PPARγ, peroxisome proliferator- activated receptor γ; S1P, sphingosine 1 phosphate.

sensor' was found to mediate ceramide-stimulated caspase 1 cleavage in macrophages and adipose tissue, and thus contribute to insulin resistance [45]. Moreover, it has been identified as a contributor to obesity-induced pancreatic damage [46].

### Adiponectin and Ceramide Deacylation

The insulin-sensitizing, antidiabetic, and cardioprotective adipokine named adiponectin elicits a broad spectrum of protective actions [47]. Remarkably, its transgenic overexpression in obese, leptin-deficient mice induces an incredible metabolic alteration whereby animals accumulate enormous amounts of adipose tissue but are protected from insulin resistance and metabolic disorders [48]. Many groups attributed these actions to its ability to activate cAMP-activated protein kinase (AMPK), a serine/threonine kinase that induces starvation responses [49]. Noting the sequence homology between adiponectin and other progestin and adipoQ (PAQR) receptors with ceramidases, the Scherer group predicted that the adipokine might elicit its broad spectrum of actions by deacylating ceramides [50]. Indeed, they found that adiponectin increased cellular ceramidase activity, and the anti-apoptotic actions of the adipokine could be negated by adding ceramidase inhibitors or by deleting a crucial residue in the predicted ceramidase motif (Figure 3, Key Figure) [51]. In these studies, eliminating adiponectin-mediated activation of AMPK had no effect on the biological actions of the adipokine [51].

## Key Figure

### Lipotoxic Actions of Ceramides

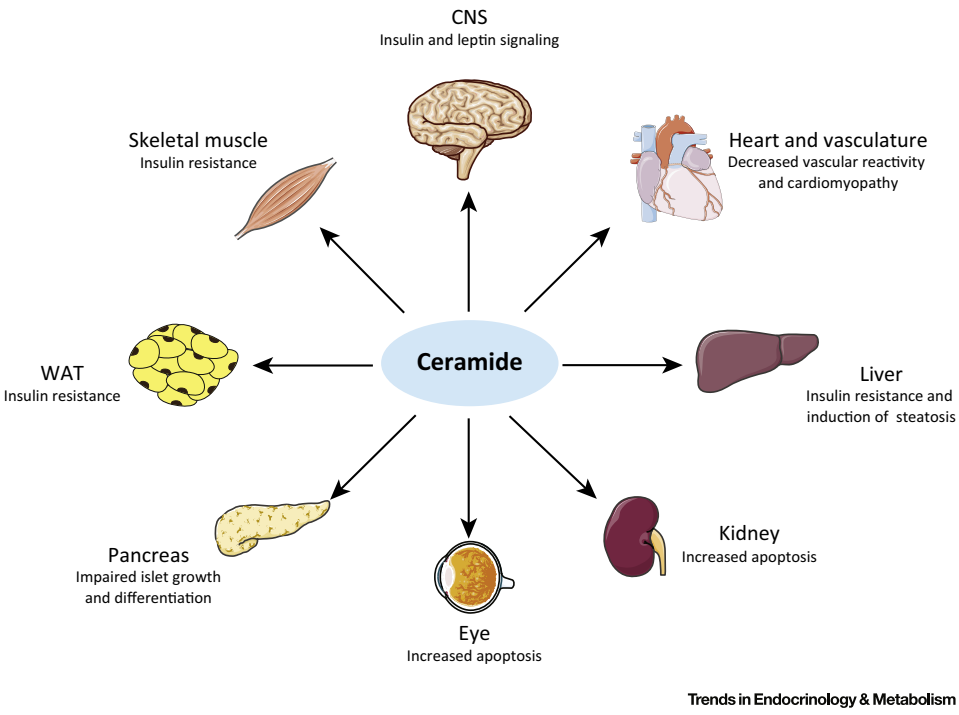


Figure 3. Ceramide accumulation in tissues has been implicated in the impairment of many metabolic processes that underlie diabetes and diabetic complications. The figure illustrates key tissues and their respective pathology associated with ceramide accumulation. Abbreviations: CNS, central nervous system; WAT, white adipose tissue; [7,11,25,45,55,63,81,94,95,109–119].

### The Fibroblast Growth Factor 21 (FGF21)–Adiponectin–Ceramide Axis

Like adiponectin, FGF21 elicits a panoply of hypermetabolic responses to increase glucose and lipid utilization [52]. The Scherer group recently proposed the existence of an FGF21–adiponectin–ceramidase axis that drives this beneficial metabolic state [51]. FGF21 was found to selectively lower ceramide levels while simultaneously inducing expression of adiponectin. Knockout of adiponectin rendered mice refractory to FGF21-mediated stimulation of metabolism and lowering of ceramide levels (Figure 3) [53].

### Farnesoid X Receptors (FXR) and the Microbiome in Ceramide Metabolism

Obesity and its metabolic comorbidities are associated with alterations in the gut microbiome. Oral treatment of lean, germfree mice with the cecal microbiota from obese animals induces hepatic triglycerides [54]. Jiang and colleagues determined that gut microbiota regulates a bile acid/intestinal FXR axis that controls ceramides levels [55]. Moreover, simultaneous administration of antibiotics selectively reduced transcripts encoding ceramide biosynthesis enzymes as well as ceramide levels in the ileum and cecum. The authors further suggested that the improvements in hepatic steatosis resulted from ceramide-mediated downregulation of hepatic SREBF1 (sterol regulatory element binding transcription factor 1/SREBP-1c) and CIDEA (cell death-inducing DFFA-like effector A), enzymes responsible for lipid synthesis and storage [55].

## Inhibition of Sphingolipid Ameliorates Insulin Resistance and Steatohepatitis

Insulin is the primary regulator of postprandial nutrient deposition, and a key feature of metabolic disorders is the resistance of target tissues to particular actions of this anabolic hormone [56]. This insulin resistance is selective because the hormone actions on uptake and storage of glucose become impaired, whereas other anabolic actions (e.g., its lipid-synthesizing effects) proceed unchecked. This condition results in a compensatory and damaging hyperinsulinemia that exacerbates hepatic steatosis, hypertriglyceridemia, and dyslipidemia by further enhancing lipid delivery to peripheral tissues, thus amplifying lipotoxicity and metabolic disease risk.

### Ceramides, Glucose Transport, and Insulin Resistance

The roles for ceramides in insulin resistance emerged from observations that it inhibited insulin-stimulated glucose transport. Mechanistically, this action of ceramide resulted from its ability inhibit insulin-mediated stimulation AKT/protein kinase B (PKB), a serine/threonine kinase that is an obligate intermediate in anabolic signaling [57–59]. Ceramide signals to AKT/PKB through two independent effectors: protein phosphatase 2A (PP2A) and protein kinase C-ζ (PKCζ) [58,60].

Using cultured cell models of insulin resistance, subsequent studies showed that blocking ceramide synthesis negated lipid-antagonism of insulin signaling. For example, pharmacological ablation and/or knockdown of genes encoding SPT (serine palmitoyl transferase), CERS (ceramide synthase), or DES1 (dihydroceramide desaturase 1) can restore insulin signaling to AKT/PKB in cultured cells bathed in excess concentrations of palmitate [10,61,62]. Overexpressing acid ceramidase to catalyze ceramide deacylation had similar effects [61].

Based on these studies, numerous groups sought to determine whether inhibition of sphingolipid synthesis was insulin sensitizing in rodents. Pharmacological inhibition of SPT, CERS, and/or DES1 prevented insulin resistance caused by lard infusion, dexamethasone, high fat feeding, and leptin or leptin receptor deficiency. These studies showed efficacy in mice, rats or hamsters [7,14,63–66]. Genetic ablation of one or more alleles of *Sptlc2* (serine palmitoyltransferase, long chain base subunit 2), *CerS6* (ceramide synthase 6), or *Des1* was also found to reduce/ablate insulin resistance in murine models [7,67,68].

An important question remaining is whether sphingolipids present in lipoproteins play a quantitatively important role in insulin resistance, as opposed to the effects always being tissue

autonomous. The Watt laboratory discovered that circulating ceramides may themselves play a large role, demonstrating that ceramides present in low-density lipoprotein (LDL) particles were sufficient to induce insulin resistance *in vitro* and *in vivo* [69]. Depletion of ceramides from the lipoprotein particles rendered them incapable of antagonizing insulin action. This interesting observation raises the possibility that ceramides packaged into LDLs in other locations (e.g., adipose tissue or the liver) may account for a large fraction of obesity-induced insulin resistance.

Glycosphingolipids encompass a class of ceramide derivatives containing one or more sugar residues. The simplest are the cerebrosides, in which ceramide is condensed with a carbohydrate moiety (i.e., glucose or galactose). Additional sugar moieties can be added to glucosylceramides to generate higher-order glycosphingolipids, including the gangliosides. Of relevance to the discussion herein, GM3 (monosialodihexosylganglioside) disrupts interactions between insulin and its receptor in adipocytes [70]. Moreover, knockout mice lacking GM3 synthase (*St3gal5*) are protected from diet-induced insulin resistance [71].

Roles for glucosylated ceramide metabolites as antagonists of insulin action are further supported by studies using next-generation inhibitors of glucosylceramide synthase (GCS). These compounds were developed for treatment of Gaucher disease, a lysosomal storage disease resulting from glucocerebrosidase deficiency, and characterized by the accumulation of glycosphingolipids. Gaucher patients were reported to be predisposed to insulin resistance [72], though verification is needed because enzyme replacement therapy actually worsens metabolic health [72,73]. GCS inhibitors have an impressive array of beneficial actions in rodents, including increased insulin sensitivity, reductions in adipose inflammation, and resolution of hepatic steatosis [74–77]. Cell culture studies confirmed that ceramides and glucosylceramides are independent antagonists of insulin action [77].

### Ceramides, Steatohepatitis, and Mitochondrial Metabolism

Nonalcoholic fatty liver disease and steatohepatitis (NAFLD/NASH) are characterized by accumulation of triglycerides in the liver owing to increased lipolysis of triglycerides in adipose tissue coupled with elevated lipid synthesis/decreased lipid oxidation in the liver [78]. The SPT inhibitor myriocin and the DES1 inhibitor fenretinide, in concert with their insulin-sensitizing effects, ameliorate hepatic steatosis [10,63,79,80]. This mechanism is unlikely to result from restoration of signaling to AKT/PKB, which is essential for insulin-stimulated lipid synthesis and storage.

A potential mechanism linking ceramides to steatosis involves its inhibition of mitochondrial electron transport chain activity. Treating mice with myriocin leads to increased mitochondrial activity [81] and enhanced oxygen consumption rates [64]. The specific culprit appears to be C16-ceramides [67,81,82]. In two independent studies, genetic interventions in CERS enzymes that modulated C16-ceramide levels altered lipid oxidation. In particular, Turpin *et al.* [67] demonstrated that knockout mice lacking *Cers6* were protected from diet-induced insulin resistance and steatohepatitis. This impressive study demonstrated efficacy following *Cers6* deletion from the entire body, liver, or brown adipose tissue. In parallel, we found that *Cers2* depletion led to compensatory increases in *Cers6* and C16 ceramides, predisposing mice to diet-induced steatohepatitis [81]. In both studies the major effect appeared to result from C16-ceramide impairment of lipid oxidation through the antagonism of electron transport chain complexes. Work in other systems supports this supposition [83].

Recent studies involving tissue-specific overexpression of acid ceramidase further reveal roles for adipose and liver ceramides in hepatic steatosis and insulin sensitivity [84,85]. Overexpression of an inducible acid ceramidase transgene in either tissue markedly resolved hepatic steatosis. This appeared to result from changes in hepatic lipid uptake as a result of

ceramide-induced translocation of the lipid transport protein CD36 to the cell membrane. PKCζ was an obligate intermediate in this newly identified ceramide action.

## Inhibition of Ceramide Synthesis Prevents β Cell Failure and Diabetes

The Unger observation that SPT inhibition prevents the destruction of β cells and the onset of frank diabetes in ZDF rats was later recapitulated with a higher-affinity and more-selective inhibitor [7]. Because these manipulations also alter insulin sensitivity, it is possibility that these effects are secondary to the improved glucose homeostasis and thus lessened demand on the β cell. An equally plausible explanation, however, is that ceramides are specific antagonists of β cell function. Ceramide has been shown to accumulate in β cells exposed to either saturated fatty acids or to a glucolipotoxic environment [86], and numerous cell-autonomous actions have been described. For example, ceramides likely contribute to the induction of apoptosis caused by palmitate, TNFα, IL-1β, interferon γ (IFN-γ), amyloid, and islet amyloid polypeptide [87]. In insulinoma cells, ceramides mediate palmitate-induced ER stress [88] and repression of insulin gene transcription [89,90].

An important area of investigation will be to determine whether tissue-specific reductions in the β cell ceramides protect animals from diabetes independently of effects in other locales.

## Inhibition of Ceramide Synthesis Ameliorates Cardiovascular Disease

The major cause of death for people with either impaired glucose tolerance or diabetes is cardiovascular disease [91]. Either owing to the roles of ceramides in glucose homeostasis, or as a result of their effects in the vasculature or heart, inhibition of ceramide biosynthesis prevents numerous cardiovascular disease endpoints.

### Ceramides and Atherosclerosis

The first studies using myriocin, the SPT inhibitor that wards off virtually all metabolic disorders in rodents, were conducted in a model of atherosclerosis, the apolipoprotein E (APOE) knockout mouse [15,92]. The treatment prevents development of plaques and enables regression of pre-formed lesions in *Apoe*$^{-/-}$ mice [15,92,93]. Ceramides derived from sphingomyelins are also implicated in plaque formation. Induction of sphingomyelin formation in the liver by sphingomyelin synthase 1 (SMS1 and SMS2) overexpression increases atherogenic potential [94], while reduction resulting from sphingomyelin synthase 1 deficiency is protective [95]. Hence, these two complementary studies implicate plasma sphingomyelin in atherosclerosis and coronary artery disease. Glucosylceramide synthase inhibitors also prevent atherosclerosis in rodent models streptozotocin [95], although this is controversial [96]. These studies strongly suggest that one or more sphingolipids contribute to atherogenic processes.

The precise identity of the deleterious sphingolipids contributing to plaque formation is not clear from these studies. The studies with the SMS knockouts suggest that sphingomyelins themselves play a role, and they very well could be bioactive and harmful. However, sphinogmyelins are the major sphingolipid in plasma and are the primary form of transport of the sphingoid backbone between tissues. A far greater number of studies have been carried out on ceramides. Ceramides certainly contribute to the dyslipidemia that underlies atherosclerosis, and these actions may account for the profound benefits associated with ceramide depletion [97]. Additional and specific roles in plaque formation have also been described. For example, acute generation of ceramide by sphingomyelinases is sufficient to induce the aggregation of lipoproteins [98,99]. Ceramide is also implicated in transcytosis of oxidized LDL across endothelial cells, thus being implicated in the retention of lipids in the vascular wall [100]. Lastly, endogenous ceramides regulate monocyte adhesion to vessel walls and subsequent promotion of LDL uptake [101].

### Ceramides, Vascular Function, and Hypertension

Hypertension results from increased abnormal constriction/dilation responses and vascular remodeling, elevating arterial blood pressure. Both myriocin and heterozygosity for *Des1* protect mice from diet-induced impairment in vascular function, negating hypertension [14]. The effects can be recapitulated in isolated blood vessels treated with palmitate, suggesting that the effect is tissue-autonomous.

One mechanism underlying the ceramide impairment of vasoreactivity involves PP2A-mediated disruption of nitric oxide synthase (eNOS) and AKT, which have strong vasodilatory functions. Ceramide inhibits phosphorylation of eNOS at key inhibitory sites, impairs endothelium-dependent vasorelaxation, increases arterial vasocontraction, and reduces NO bioavailability in endothelial cells [14,102]. Another mechanism linking ceramides to vascular function includes decreased membrane fluidity, which is reduced in hypertensive rats. Extensive intermolecular hydrogen bonding between ceramides increases membrane rigidity [103]. Other sphingolipid species have also been reported to have vasoactive roles either by stimulating contraction or impairing endothelium-dependent relaxation [104,105]. Bolz *et al.* [106] found that overexpressing sphingosine kinase type 1 in vascular smooth-muscle cells of resistance arteries increased both resting tone and myogenic responses, while overexpression of a dominant-negative sphingosine kinase inhibited these processes.

### Ceramides and Lipotoxic Cardiomyopathy

Lipotoxicity likely contributes to cardiac dysfunction in (i) obesity-related and (ii) diabetic cardiomyopathy, and pathological assessments reveal lipid accumulation in these specimens. Moreover experimental manipulations that promote cardiac-specific lipid accumulation in mice induce cardiac dysfunction [107]. In a mouse model of cardiac lipotoxicity induced by heart-specific overexpression of lipoprotein lipase, both myriocin and heterozygosity for *Sptlc2* improved cardiac function and corrected cardiac hypertrophy [19]. Russo and colleagues specifically implicated C16-ceramides produced by CERS5 [108]. However, cardiac-specific deletion of both alleles of *Sptlc2* led to abnormal heart development [17], making it difficult to ascertain whether the ceramides that participated in cardiolipotoxicity were generated within the cardiomyocyte. Ceramide mechanisms relevant to cardiac dysfunction include the aforementioned apoptosis, inhibition of insulin signaling, and ER stress [107].

## Ceramides and Diabetic Complications

Far less work has been done investigating ceramide actions in other diabetes complications, but the nascent studies suggest potential roles (Figure 3). Several groups have highlighted an action of ceramides in mesengial cell apoptosis, an event crucial for the development of diabetic nephropathy [109]. SPT expression is elevated in renal tubular epithelial cells isolated from diabetic patients, and inhibition of ceramide synthesis ameliorates tubular epithelial cell death [109]. Some studies have shown roles for glycosphingolipids in diabetic nephropathy [110,111], and further studies have suggested roles for ceramides in retinal pericyte apoptosis and in the development of diabetic retinopathy [112,113].

## Concluding Remarks and Future Perspectives

Even using the most conservative estimates, the worldwide burden of diabetes and cardiovascular disease is staggering. Approximately one in every 12 people is diabetic, and an estimated 387 million people have the disease (www.idf.org). For every person that is diabetic, another person has impaired glucose metabolism (one in five), which places him or her at risk for heart disease and stroke. The evidence identifying ceramides and ceramide metabolites in these disorders is substantial, and enzymes regulating their synthesis, degradation, or actions are inviting therapeutic targets.

### Outstanding Questions

Which tissues are most sensitive to ceramides, and which tissues produce the ceramides that impair metabolic homeostasis?

Of the many ceramides and ceramide metabolites that accumulate in cells, which ones impair tissue function?

How are small changes in sphingolipid concentrations sensed inside the cell?

Why have the correlational studies evaluating ceramide levels in relation to metabolic endpoints been so discordant?

Does targeting ceramide biosynthesis in humans elicit the same spectrum of beneficial metabolic effects? Could this be a viable therapeutic strategy for combating prevalent metabolic pathologies?

Key questions remain (see Outstanding Questions). Though several mechanisms have been identified to explain ceramide action (e.g., inhibition of AKT/PKB, oxidative phosphorylation, lipid or lipoprotein transport, etc.), they are unlikely to explain the full array of benefits derived from ceramide depletion. Moreover, the cellular means of sensing small changes in ceramides to initiate large metabolic adaptations is unclear and wholly unsatisfactory. In reality, the precise, tissue-specific roles of ceramides in cellular function have largely been limited to *in vitro* systems, and tissue-specific roles need to be investigated in more biologically-relevant contexts. Fundamental discovery biology investigating the role of these lipids in cellular function continues to be of primary importance for the development of new therapies and understanding the etiology of metabolic disorders.

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