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Literature Review

Ceramide dependent lipotoxicity in metabolic diseases

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Abstract. Sphingolipids, a major class of lipids in cell membranes, play diverse roles in biology. They are synthesized by a highly conserved biosynthetic pathway that leads to the production of ceramides, the major precursors of most complex sphingolipids. Almost all known stress stimuli including inflammatory agonists, chemotherapeutics, and saturated fatty acids induce the synthesis of ceramide and its metabolites. A panoply of recent studies has implicated ceramides in the development of the metabolic comorbidities of obesity such as diabetes and cardiovascular diseases. In particular, inhibition of ceramide biosynthesis in rodents ameliorates insulin resistance, diabetes, cardiomyopathy, atherosclerosis, and steatohepatitis. These data implicate ceramides as major contributors to the development of metabolic diseases. This review summarizes recent findings on this emerging class of bioactive lipids with an emphasis on studies using *in vivo* models to understand their role in metabolic disease.

1. Introduction

The majority of obese individuals develop insulin resistance, a condition characterized by impaired cellular responses towards insulin [1]. If not controlled, prolonged insulin resistance increases risk for Type 2 Diabetes and cardiovascular disease [2]. A large body of evidence suggests that lipid-induced insults (lipotoxicity) in metabolic tissues drives the initiation and progression of insulin resistance, diabetes and metabolic disorders [3].

As fatty acids enter cells, they are rapidly converted to acyl-CoA's before undergoing one of three metabolic fates. They can be coupled to (a) glycerol to produce glycerolipids (e.g. triacylglycerol, diacylglycerol, phosphatidylcholine, etc.), (b) carnitine for delivery into mitochondria to produce acyl-CoA for ATP production or cholesterol synthesis, or (c) serine to generate sphingolipids (e.g. ceramide, sphingomyelin). Sphingolipids are the least abundant (∼20% of glycerolipids) [4, 5]. However, when they accumulate above a critical threshold, they impair insulin action in adipose tissue, skeletal muscle, and/or the liver [6, 7] and modulate energy metabolism [7, 8]. Moreover, adiponectin appears to elicit its anti-diabetic and cardioprotective actions by activating receptors with intrinsic ceramidase activity to degrade ceramides [9, 10]. In addition, saturated fatty acids (SFAs) induce their antagonistic effects on insulin signaling in peripheral tissues, such as the skeletal muscle, by enhancing activation of TLR-4 receptor signaling to increase biosynthesis of ceramides [6]. In this review, we provide a perspective on *in vivo* studies implicating ceramides in the development of metabolic diseases.

2. Ceramide synthesis and metabolism

Although ceramides are prevalent in the diet, they are largely degraded in the mammalian intestine [11]. Their production in animal tissues is driven by a conserved *de novo* ceramide synthesis

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pathway which begins in the endoplasmic reticulum with the condensation of palmitoyl-CoA and serine, catalyzed by the enzyme serine palmitoyltransferase (*Spt 1-3*), to produce 3-ketosphinganine (Fig. 1) [12]. Three subsequent reactions follow: 3-ketosphinganine reductase (*3Ksn*) generates sphinganine, which is then *n*-acylated by (dihydro) ceramide synthase (*Cers 1–6*) to produce dihydroceramide. Then, desaturases (*Des1* and *2*) introduce a distinctive double bond in dihydroceramide to produce ceramides. The diversity in the sphingolipid family results from a family of mammalian *Cers* (*Cers1–6*), which add fatty acids of different chain lengths to the sphingoid backbone, leading to the ultimate generation of ceramide with variable acyl chain lengths ranging from 14-carbon to 34-carbon atoms (Fig. 1) [13]. Importantly, ceramides with varying acyl chain compositions are generated in specific tissue and cell types depending on the physiological and pathological state which show differential effects on the development of metabolic diseases [14, 15]. The double bond introduced by dihydroceramide desaturase imparts many of ceramide's unique biophysical properties [12].

3. Regulation of ceramide production in obesity

The question as to how and when ceramides accumulate in obesity has attracted considerable attention. The initial assumption was that increased supply of the substrates palmitate and serine from overnutrition was the major source of tissue ceramides in obesity. However, recent studies have revealed that tissue ceramides are also regulated by hormonal cues, which modulate rates of ceramide synthesis and degradation [6, 16]. In this section, we will present evidence gained over the years that has led to the refinement of the initial hypothesis.

3.1. Ceramides in obesity induced inflammation

Obesity is associated with chronic low grade inflammation characterized by increased recruitment and activation of macrophages to adipose tissue, resulting in augmented expression and secretion of inflammatory cytokines [17, 18]. These inflammatory cytokines (e.g. TLR4 agonists, TNF-α, interleukins, etc.) all increase levels of sphingolipids, generally without affecting glycerolipids [19]. The prevailing wisdom is that these inflammatory triggers work in concert with excessive nutrient availability to drive sphingolipid production. Circulating inflammatory cytokines consistently show a particularly tight association with circulating ceramides and insulin resistance [19, 20].

Several findings support the involvement of the innate immunity receptor toll-like receptor 4 (TLR4), which is either stimulated or amplified by saturated fats, as an important modulator of lipid-induced insulin resistance and ceramide synthesis [6]. Indeed, the preponderance of data reveal that TLR4-induced ceramide synthesis is an essential component of fat-induced insulin resistance. Lipopolysaccharide (LPS), a TLR4 agonist, selectively upregulates *de novo* ceramide synthesis. Moreover, mice lacking TLR4 fail to accumulate ceramide in the presence of elevated saturated fatty acids (Fig. 1) [6, 21, 22]. These data establish TLR4 signaling as an essential component linking saturated fats to the modulation of ceramide synthesis and anabolic metabolism. Mechanistically, these effects are partially mediated by activation of the Nod-like receptor (Nlrp3) inflammasome, which senses ceramides to induce caspase-1 cleavage in macrophages and adipose tissue, and by contributing to the development of insulin resistance by inhibiting AKT activation [23, 24].

3.2. FGF21-adiponectin-ceramide axis

The adipokine adiponectin has received considerable attention for its potential anti-diabetic actions. Adiponectin regulates glucose and lipid homeostasis through actions in the liver, adipose, and pancreatic tissue [25–27]. In addition, adiponectin regulates lipid spillover into non-adipose tissue by governing rates of lipid synthesis, oxidation and lipolysis, as well as by inhibiting inflammation. These beneficial effects of adiponectin were previously thought to be mediated by AMPK, a serine/threonine kinase [25]. However, the Scherer group has recently demonstrated that adiponectin receptors AdipoR1 and 2 stimulate deacylation of ceramide, yielding sphingosine that can be converted into sphingosine 1-phosphate (S1P) by sphingosine kinases [9]. The resulting sphingosine and/or S1P prevent apoptosis of pancreatic β-cells and cardiomyocytes and exert an anti-diabetic effect. Moreover, once synthesized, S1P is transported to the extracellular environment and binds to the S1P receptors to activate AMPK [9]. Consistent with this, Tanabe et al. initially showed that crystal structures of

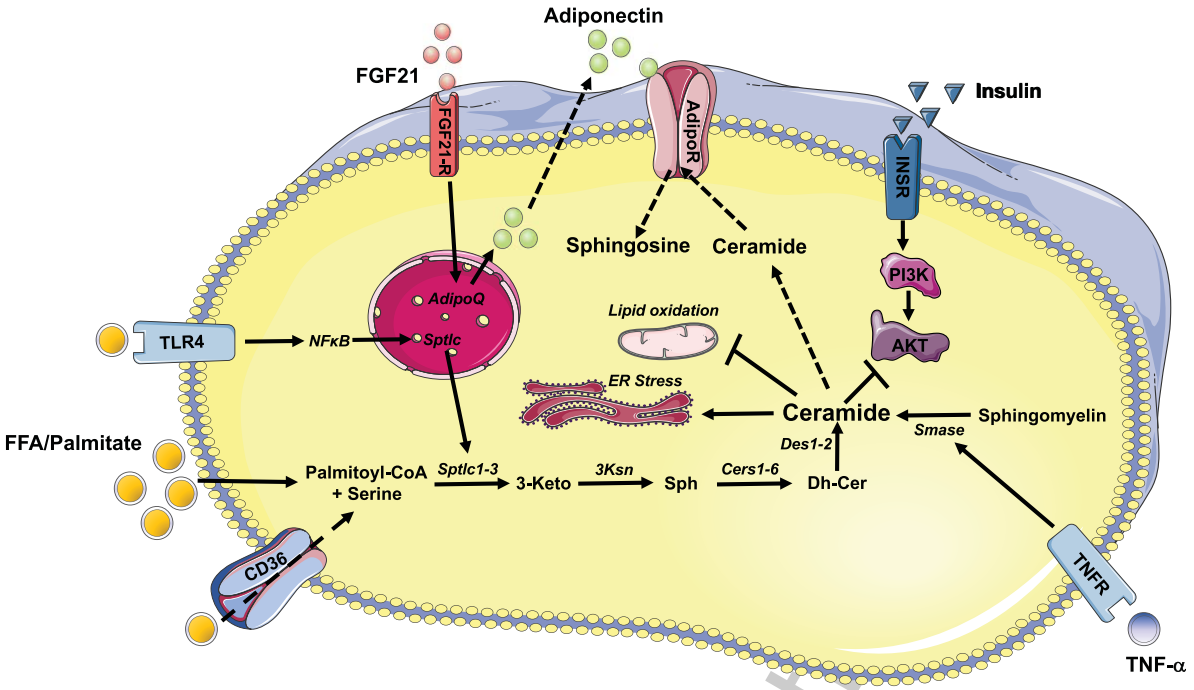

Fig. 1. Schematic illustration of ceramide synthesis and its action in metabolic tissues. Free fatty acids, palmitate and inflammatory agonists stimulate the synthesis of ceramides. Excess accumulation of ceramides in insulin responsive tissues inhibits AKT/PKB resulting in reduced insulin response. In addition, ceramides, elicit its deleterious effect by inhibiting mitochondrial function and inducing ER stress. Inhibition of ceramide synthetic pathway improves insulin sensitivity. Similarly, FGF21 and adiponectin exhibits its beneficial effect partially by regulating rates of conversion of ceramide to sphingosine. Abbreviations of enzymes: *Cers*: Ceramide synthase; *Des*: desaturase; *Ksn*: 3-ketosphinganine reductase; *Smase*: sphingomyelinase; *Sptlc*: serine palmitoyltransferase.

human AdipoRs possess a hydrophobic binding pocket potentially resembling that of the ceramidases [28]. More recently, Vasiliauskaite-Brooks et al. showed that purified adiponectin receptors possess inherent ceramidase enzymatic activity (Fig. 1) [10]. Moreover, they solved the crystal structure in the presence of ceramide, obtaining a final entity bound to a fatty acid product of the reaction [10].

FGF21, a member of fibroblast growth factor (FGF), has garnered a considerable amount of attention because of its ability to modulate glucose and lipid homeostasis and whole-animal energy utilization [29, 30]. In a series of elegant studies, Scherer and colleagues recently demonstrated that FGF21 stimulates adiponectin secretion in rodents, thereby decreasing ceramide levels. Interestingly, the deletion of adiponectin renders rodents' refractory to FGF21, at least with regards to its effects on ceramide levels and energy metabolism. Collectively, these studies demonstrate the presence of an FGF21-adiponectin-ceramide axis that modulates glucose and energy homeostasis (Fig. 1) [31].

3.3. Ceramide, gut microbiota, and obesity

Oral transplantation of cecal microbiota derived from obese mice into lean germ free mice leads to an increase in hepatic triglyceride content [32], which demonstrates that alteration of the gut microbiota contributes to the development of obesity and its comorbidities. However, the mechanisms linking gut microbiota to metabolic homeostasis have been elusive. Recently, Gonzalez and colleagues found that gut microbiota regulate a bile acid/intestinal FXR axis to alter ceramide pathways, which may led to hepatic triglyceride accumulation [33]. Moreover, their study goes on to demonstrate that depleting the gut microbiota with antibiotics reduces transcripts encoding for the genes involved in ceramide biosynthesis in the ileum and cecum and lowers serum ceramide levels, an effect mediated by the intestinal FXR receptor. The authors further present data suggesting that improvements in hepatic steatosis result from FXR dependent downregulation of ceramide and hepatic *Srebp1c* and *Cidea* [33]. Their work further demonstrated that intestinal FXR modulates ceramides content in

the gut to reduce hepatic mitochondrial acetyl-CoA levels and pyruvate carboxylase activities, thereby attenuating hepatic gluconeogenesis [34].

4. Ceramides influence glucose homeostasis

Substantive evidence accumulated over the past decade has convincingly demonstrated that sphingolipids, especially ceramides and its metabolites, are key mediators of insulin resistance and related metabolic comorbidities [35]. Early studies identifying roles for ceramide in insulin resistance came from direct application of ceramide analogs to isolated skeletal muscles and cultured adipocytes [36, 37]. These studies revealed that ceramide inhibits insulin-stimulated glucose uptake and glycogen synthesis [38]. Subsequent to that, implementation of pharmacological and genetic strategies to inhibit synthesis of ceramide or glucosylceramides in rodent models of obesity was shown to increase insulin sensitivity [35]. Moreover, profiling studies revealed an inverse relationship between ceramides and insulin sensitivity in rodents, non-human primates and humans [39, 40]. The strength of the relationship is particularly strong when inflammation is considered in concert [6, 22, 41]. Mechanistically, cell-autonomous ceramide accumulation has been shown to inhibit AKT/PKB phosphorylation by activating protein phosphatase 2A, and blocking the translocation of AKT/PKB to the plasma membrane through PKCζ activation (Fig. 1) [42–44]. Studies using lipid infusion or isolated muscles reveal that ceramides are obligate intermediates linking saturated fatty acids, but not unsaturated ones, to the development of insulin resistance [6, 45–47].

In rodents, manipulation of ceramide synthesis or degradation pathways through pharmacologic or genetic means have profound effects on modulating insulin sensitivity [35]. Importantly, pharmacological inhibition of the ceramide biosynthetic enzymes SPT or DES1 using myriocin or fenretinide, respectively, elicits dramatic improvements in insulin action and glucose homeostasis in a high fat fed mice, fructose fed hamsters, leptin or leptin receptor deficient rats or mice, and dexamethasone treated rats or mice. [7, 48–50]. Moreover, mouse models bearing haploinsufficiency of either *Sptlc2* or *Des1,* which are essential for ceramide synthesis, show substantial improvements in insulin sensitivity when exposed to high fat diet and/or dexamethasone [7, 51]. Another approach to reduce ceramide levels in rodents involves the overexpression of acid ceramidase, which converts ceramides into sphingosine. In cultured cells, the transgene negated the inhibitory effects of palmitate on insulin signaling [42, 45]. This approach was also efficacious *in vivo*, as overexpression of ceramidase in adipose tissue or the liver resolved impaired glucose tolerance [52].

Researchers are starting to obtain greater clarity on the influence of acyl chain length on ceramide action. Much of the work comes from cells or animals lacking one of the six-ceramide synthase enzymes (*Cers1–6*) that catalyze the n-acylation of sphinganine [13]. Studies involving the ablation of *Cers2* and *Cers6* in mice came to the common conclusion that C_{16}-ceramides contributed to insulin resistance [14, 15]. First, the Brüning group demonstrated that genetic deletion of *Cers6*, the enzyme that adds the C_{16}-acyl-chain, protects mice from HFD-induced obesity, glucose tolerance and insulin resistance [14]. Second, Summers and colleagues demonstrated that haploinsufficiency for *Cers2* reduced C_{24}-ceramides, but elicited a compensatory increase in C_{16}-ceramides. The elevation of C_{16}-ceramides led to impairments in glucose tolerance and insulin sensitivity [15]. Mechanistic studies suggest that the C_{16}-ceramides impair metabolic homeostasis by inhibiting mitochondrial $\beta-$oxidation. Interestingly, genome wide association studies have identified a common *Cers2* polymorphism, introducing a single amino acid substitution at position 115, that is strongly associated with insulin resistance [15, 53].

4.1. Ceramides in adipose tissue

We recently completed a study doing a careful analysis of the role of ceramides in adipose tissue *in vivo*. The work was an offshoot of our studies with myriocin, a potent inhibitor of the enzyme SPT isolated from the fungus *Isaria Sinclairii*. The reagent has been used in a series of studies to reduce ceramides in rodents, which ameliorates insulin resistance and various other metabolic disorders in obese mice, rats, and hamsters [7, 48, 49, 54–57]. We found that it induced a broad spectrum of changes in the adipose bed including reduced adipocyte size, increase recruitment of M2 macrophages, and elevated numbers of brown/beige adipocytes in white adipose tissue, particularly in the subcutaneous depot [58]. We then found that adipose-specific ablation of *Sptlc2* recapitulated the effects of myriocin including the improvement in insulin sensitivity and glucose

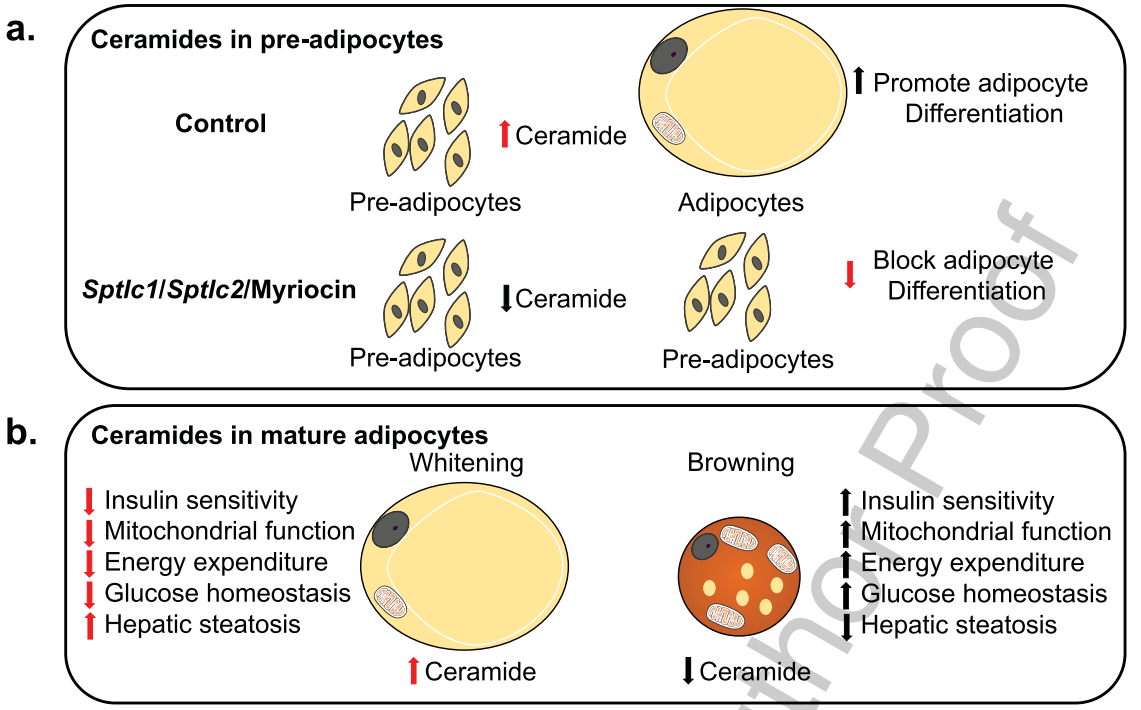

Fig. 2. Model for Ceramide sensing in adipocytes and its systemic effects. (a) Ceramides are essential for the differentiation of pre-adipocytes into adipocytes. Ablation of *Sptlc1/2* and pharmacological inhibition of ceramides biosynthesis in pre-adipocytes blocks differentiation. (b) Adipocyte ceramides serve as nutritional determinants to promote lipid storage and inhibit thermogenic capacity hence promoting "whitening" rather than "beigeing/britening" of adipocytes. Ablation of ceramide synthesis in mature adipocytes of obese mice promotes beigeing/britening of adipocytes which improves insulin sensitivity and mitochondrial function in adipocytes and has systemic effects on improving mitochondrial function, energy expenditure, glucose homeostasis and resolving hepatic steatosis.

tolerance, resolution of hepatic steatosis, recruitment of beige adipocyte in the adipose tissue, and improved mitochondrial function [58]. These adipose-specific changes were sufficient to increase whole-body energy expenditure. Based on these findings, we proposed that ceramides act as nutrient signals that direct the adipocyte towards a hypo-metabolic, rather than thermogenic phenotype (Fig. 2).

In congruence with these findings, Jiang et al. demonstrated that ectopic ceramides inhibit the browning of beige adipocytes, suggesting that endogenous ceramides could be autonomous regulators of adipocyte function [59]. We applied a similar approach in the aforementioned study, using various pharmacological reagents to manipulate levels of endogenous ceramides. Collectively, the work shows that the actions on the adipocyte were cell-autonomous and driven by ceramides, but not other sphingolipids.

Scherer and colleagues [52] used another approach to selectively reduce adipose ceramides. Specifically, they overexpressed acid ceramidase (*Asah1*) in adipose tissue. Though they did not report effects on adipose tissue browning/beiging, transgene induction in adipose tissue quickly (i.e. within 3 days) resolved hepatic steatosis and improved glucose tolerance, an effect that was similar to that observed with *Sptlc2* ablation in adipose tissue [58].

Of note, recent papers by the Proia and Park laboratories found that ablation of either *Sptlc1* or *2,* respectively, in adipose tissue impaired adipose differentiation and elicited a lipodystrophic phenotype [60, 61]. In these studies, the authors used an adiponectin-Cre-recombinase line from Jackson Laboratories that expresses the transgene earlier in development [62]. We hypothesize that this accounts for the difference in phenotype. In concordance with their work, our studies in primary cells show that myriocin is a potent inhibitor of adipocyte differentiation [58].

Adipose tissue preferentially expresses a ceramide synthase (i.e. *Cers6*) that makes the deleterious C_{16}-ceramides. Associations between adipose C_{16}-ceramides and metabolic dysfunction have been observed [14]. Moreover, *Cers6* expression is dramatically increased in obese individuals [14]. Turpin et al.

generated mice lacking *Cers6* in brown adipose tissue [14]. BAT-specific inhibition of *Cers6* resolved hepatic steatosis, improved glucose tolerance, and enhanced mitochondrial β-oxidation and energy expenditure. These studies further highlight the importance of ceramide accumulation in BAT in regulating systemic metabolic homeostasis.

5. Ceramides in the liver

Nonalcoholic fatty liver disease is characterized by an increased accumulation of triglycerides in hepatocytes. The condition is a major health problem that predisposes individuals to cardiovascular disease, liver cancer and cirrhosis [63]. The condition results from insulin resistance in adipose tissue, leading to increased lipolysis that liberates fatty acids destined for the liver, causing impairments in hepatic lipid oxidation. [64]. In mice and humans, the degree of hepatic steatosis and/or insulin resistance positively correlates with hepatic ceramides [65, 66]. Pharmacological inhibition of SPT by myriocin or DES1 by fenretinide in rodent models of obesity reduces hepatic lipid accumulation [49, 50].

The aforementioned studies on the CERS enzymes again support roles for C_{16}-ceramides in promoting fat deposition in the liver via impaired lipid oxidation. In particular, Turpin et al. [14] demonstrated depletion of *Cers6* from the liver protected obese mice from steatohepatitis and insulin resistance. Conversely, *Cers2* depletion led to compensatory increases in *CerS6* and C_{16}-ceramides and predisposed mice to diet-induced steatohepatitis [15]. A subsequent study showed that mice lacking *Cers5,* which also contributes to C_{16}-ceramide synthesis, exhibit reduced C_{16}-ceramide content in liver, improved glucose metabolism and insulin sensitivity, and protection from hepatic steatosis [67].

The Scherer lab used the aforementioned system enabling inducible expression of acid ceramidase to study the role of ceramides in the liver. Overexpression of acid ceramidase (*Asah1*), which results in decreased hepatic ceramides, protected the animals from hepatic steatosis and improved insulin sensitivity [52]. This protection appeared to result from changes in hepatic lipid uptake, as they found that ceramide-induced translocation of the lipid transport protein CD36 to the cell membrane. PKCζ was an obligate intermediate in this newly identified ceramide action. Similarly, in liver specific inducible overexpression of adiponectin receptor (AdipoR), adiponectin decreased hepatic ceramide content, improved hepatic insulin resistance and protected against hepatic steatosis by increasing AdipoR-induced ceramidase activation [68].

6. Ceramides in muscle

Despite the abundance of data from interventional studies indicating that ceramides improve insulin sensitivity, the relative importance of ceramides as regulatory factors in skeletal muscle metabolism has been contentious. The controversy stems from discordance in lipidomic profiling studies, as some groups have shown strong associations between muscle ceramides and insulin resistance [69–76], while others have found no such relationship [77–80]. Indeed, this issue has been discussed further in a recently published Crosstalk "debate" sponsored by the Journal of Physiology where investigators took oppositional positions about the roles for ceramides as modulators of muscle function [81, 82]. Despite this contention, the initial *in vitro* studies convincingly demonstrated that increasing ceramide content in skeletal muscle cells potently inhibits insulin signaling [42, 83, 84]. Conversely, pharmacological inhibition of ceramide synthesis or increased degradation of ceramides in myotubes, attenuates palmitate induced inhibition of insulin signaling [85]. Moreover, inhibiting ceramide synthesis was shown to negate palmitate-induced insulin resistance in isolated muscle strips and lipid-infused rodents [6, 7]. Nonetheless, muscle-specific manipulations of ceramide content have not been conducted. To further hone the tissue-specific role of ceramides in muscle, these studies should be pursued in the future.

7. Ceramides in beta cells

Blocking ceramide production prevents the destruction of beta cells in rodent models of type 2 diabetes. Whether this is due to autonomous actions within the beta cell or is a consequence of its insulin-sensitizing properties is unclear. However, studies in cultured cells suggest that ceramide may have autonomous actions within the cell type. In particular, fatty acids have been shown to impair insulin secretion and insulin gene transcription, in addition to inducing apoptosis. The chronic adverse effects of FFAs on β-cell function and viability are

potentiated in the presence of hyperglycemia, a phenomenon that has been termed gluco-lipotoxicity [24]. Ceramide has been shown to accumulate in β-cells exposed to either saturated fatty acids (lipotoxicity) or to hyper-physiologic glucose environment [86]. Moreover, ceramides are capable of inhibiting insulin gene expression, blocking proliferation, and inducing apoptosis both in mouse and human islets [87–96]. Glycosylated derivatives of ceramide (i.e. gangliosides) have been identified as putative antigens that contribute to the auto-immune response [97–100]. These *in vitro* studies suggest that ceramides could contribute to the decline in β-cell function that underlies diabetes. However, their roles *in vivo* (e.g. with tissue specific knockouts) have not been studied in sufficient detail and are essential for delineating the roles of ceramides in β-cell function.

8. Ceramides in central nervous system

Data gleaned over the past decade have established the role of hypothalamic insulin and leptin signaling in modulating energy and glucose homeostasis [101, 102]. In particular, the Clegg laboratory found that introducing saturated fatty acids into the brain disrupts insulin signaling in the hypothalamus at the level of AKT/PKB [103]. In addition, the Summers group reported that ceramides accumulate in hypothalamus following either high fat diet feeding or acute lipid infusion [6]. Taken together these studies raise the interesting possibility that ceramides accumulate in the hypothalamus to modulate energy homeostasis. In support of this hypothesis, Contreras and colleagues recently demonstrated ceramide induced lipotoxicity in the hypothalamus modulates weight gain by reducing brown adipose tissue thermogenesis [104].

Additional studies suggest that glucosylated ceramides within the CNS may modulate peripheral metabolism. In particular, neuronal expression of glucosylceramide synthase (GCS), which regulates the synthesis of ceramide metabolite glucosylceramide, has also been implicated as a modulator of body weight and energy homeostasis [105]. Mice deficient in glycosphingolipids in the hypothalamus developed progressive obesity and displayed a decrease in sympathetically mediated thermogenesis [105]. Moreover, rAAV-mediated *Ugcg* (encoding for GCS) delivery to the hypothalamic arcuate nucleus led to ensuing elevations in nuclear glucosylceramides which ameliorated obesity. Mechanistically, GCS-depleted neurons displayed inadequate leptin receptor [42] activation, requiring neuronal gangliosides GM1 and GD1a to be recruited to the ObR upon ligand stimulation [105]. Although these observations suggest the essential requirement of GCS in regulating food intake, we cannot distinguish whether the obese phenotype in this animal model results from the lack of glucosylceramides or the accumulation of ceramides.

Although nascent, studies accumulated in recent years have refined our understanding of ceramide-mediated lipotoxicity in the hypothalamus in regulating energy homeostasis. These data raise interesting questions as to which orexigenic signals are modulated by ceramides or its metabolites.

9. Ceramides in the heart and vasculature

An estimated 65% of people that die a cardiovascular death have either impaired glucose tolerance or diabetes [106]. Interestingly, inhibition of ceramide biosynthesis shows beneficial effects in several rodent models of cardiovascular diseases, including atherosclerosis, hypertension and cardiomyopathy [107–110]. Of note, ceramide has been identified as surrogate biomarkers that predict cardiovascular events, and clinical tests are being made available to patients [111].

Whether this protective effect of ceramide depletion interventions is due to improvements in glucose homeostasis or a result of autonomous effects in the vasculature or heart is unclear [24]. For example, the atherogenic effects of ceramide could also be due to autonomous effects on the vessel wall. Ceramides also induce transcytosis of oxidized low-density lipoproteins across endothelial cells, leading to the retention of lipids in the vascular wall [112] and promote monocyte adhesion to vessel walls [113], which provides a mechanism that could contribute to plaque formation. Furthermore, vascular dysfunction critically underlies cardiovascular diseases, including hypertension. Both myriocin and haploinsufficency for *Des1* protect mice from diet-induced impairment in vascular function, negating hypertension [114]. Studies in isolated vessels exposed to palmitate imply that ceramides may also have autonomous actions in the vessel [114]. Ceramide was an obligate intermediate linking palmitate to the impairment in vascular reactivity. These effects were due to ceramide induced co-localization of protein phosphatase 2A

(PP2A) with eNOS, leading to the inhibition of eNOS phosohorylation and its dissociation from AKT/PKB [114].

Obesity is associated with an increased incidence of cardiac dysfunction and cardiomyopathy that contributes to morbidity and mortality from cardiac infarction. Ceramides have been found in pathological specimens and worsen lipotoxicity induced cardiomyopathy [115]. Pharmacological or genetic inhibition of ceramide synthesis in a model of lipotoxic cardiomyopathy (i.e. cardiac specific overexpression of human lipoprotein lipase) abrogates apoptosis and cardiac contraction [115]. However, heart-specific deletion of *Sptlc2* impairs cardiac function [116], making it difficult to ascertain whether ceramides that regulate cardio-lipotoxicity were generated within the cardiomyocyte.

10. Conclusion

The redundancy of approaches utilized in rodent models so far strongly suggests that therapeutic strategies that reduce pathological ceramides should improve insulin sensitivity and help patients achieve better glycemic control. Moreover, such clinical interventions should delay or prevent the various comorbidities of obesity, such as diabetes and heart disease. Nonetheless, a number of questions still remain [24, 117]. Firstly, our understanding of the tissue-specific roles of ceramides in disease etiology is still not fully defined. To this end, the advent of novel mouse tools enabling tissue-specific manipulation of ceramides will help to identify which tissues are most sensitive to ceramide accumulation. Secondly, though initial studies identified a couple of key mechanisms (i.e. regulation of AKT) for ceramide actions, the plethora of effects elicited by ceramide seems to be unlikely to be fully explained solely by this PP2A-AKT axis. Identifying additional molecular mechanisms will be crucial for understanding the roles of ceramides. Thirdly, ceramides are intermediate metabolites of the complex sphingolipids which are not in a static state and have a high degree of turnover. A better understanding the regulatory nodes in the ceramide biosynthetic pathway that are modulated during metabolic abnormalities could lead to the identification of better therapeutic targets. Despite these questions, the data thus far obtained place ceramides at the nexus of a nutrient signaling network that has profound effects on a wide variety of metabolic disease processes.

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